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K. VAS

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E. ALMÁSI, R. LÁSZTITY,
K. LINDNER, P. SPANYÁR

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Edited by

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PREFACE

Parallel with the recent rapid expansion of the country's food industry, Hungarian research in food science and technology grew in volume and quality. At present some three hundred scientists in sixteen research institutes, three university departments and a university college are working on the physico-chemical, chemical, biological, microbiological, enzymological, engineering, instrumentation and economic aspects of the production, storage, preservation and quality evaluation of foods.

In recognition of the importance and scientific achievements of the above activities, the Sections of Chemistry and of Agronomy of the Hungarian Academy of Sciences have recently established a Joint Committee for Food Science and accepted the latter's proposal to launch a new journal devoted to the above subjects under the title "Acta Alimentaria Academiae Scientiarum Hungaricae".

On behalf of the Editorial Board, I take great pleasure in presenting the first issue of this new periodical in the hope that it will lend itself for giving some insight into Hungarian food research and also that its communications will contribute to the world-wide development of food science.

K. Vas
Editor

Acta Alimentaria, Vol. 1 (1), pp. 5—16 (1972)

EFFECT OF RADURIZATION DOSES ON POLYPROPYLENE FOIL

I. VARSÁNYI, I. KISS and J. FARKAS

(Received June 29, 1970)

Packaging materials of radiation-treated food products should, beyond technical and economic requirements, meet also hygienic and quality criteria.

"Moplefan BT" type polypropylene foil, used as packaging material for radiation-preserved pork cuts, was examined for eventual changes of structure or mechanical properties caused by gamma-radiation. Samples of foil exposed to 0, 50, 100, 200, 400, 600, 700 and 800 krad were investigated by infra-red and ultraviolet spectrophotometry and tested for tensile strength and elongation.

Evaluation of the infra-red and ultraviolet spectra showed statistically significant change in both infra-red light transmission and ultraviolet light absorption due to structural modification of the foil upon exposure to about 800 krad. Nevertheless, from the practical angle this change was relatively slight, being in the range of 5—10%.

Exposure at dose levels up to 600 krad did not significantly alter the tensile strength of the foil, however 700—800 krad already effected a highly significant, about 20% reduction of the tensile strength. A notable increase of elongation at rupture was observed already at a dose level of 400 krad.

The experimental results suggest that non-oriented polypropylene foil, treated on one side for printing purposes is suitable for the packing of meat treated with radurizing (radiation-pasteurizing) doses of gamma-radiations, as dose levels of 800 krad or less did not significantly alter the proportion of the atactic-isotactic regions. Thus, changes of the other characteristic properties could not be important either.

The packaging of food products is an important field of modern food industry. Appropriate packaging of commercial foodstuffs is not only an economic, but also a hygienic requirement. The well-packed products usually have a longer shelf life. And at the same time, packaging prevents recontamination of the finished food product, extending thereby its keeping quality and palatability.

Requirements for storage conditions vary with the nature of the food product. Packaging materials should always be selected for the specific purpose of protecting the product against mechanical, chemical and biological effects. Another factor to be considered is the eventual interaction between product and packaging material which may result in deterioration of either the former or the latter as well as in alteration of the product characteristics or diminution of storage life, sensory qualities, palatability or nutritional value.

Investigations into the value of radiation preservation in the food industry have necessitated the study of the radiation sensitivity of packaging

foils. In certain polymer systems the irradiation has been shown to cause structural changes by breaking, transposal or formation of chemical bonds (BLACK & LYONS, 1957). Such structural modifications may change the properties of polymers, by rendering them less resistant to mechanical effects, permeable to microorganisms, etc.

The present studies were carried out to clarify whether the radiation preservation of pork cuts, packed in polypropylene pouches, caused changes in the packaging material. In other words, it was investigated whether the radiation dose considered by the authors sufficient for the preservation of pork had modified the properties of the polypropylene foil.

1. Materials and methods

1.1. Test material and its preparation

Boned and cut pork chops were packed one by one into pouches of 150×180 mm of "Moplefan BT" (MONTECATINI & EDISON) polypropylene foil of 0.05 mm thickness, with as little headspace as possible and sealed. Prior to radiation treatment the samples were stored for 16—18 hours at 6—8 °C.

1.2. Radiation treatment

A ^{60}Co gamma radiation source of 80,000 Ci nominal activity (Institute for Isotopes, Hungarian Academy of Sciences) was used for radiation treatment. The samples were irradiated at 20—25 °C temperature at the following dose levels: 0.05, 0.10, 0.20, 0.40, 0.60, 0.70 and 0.80 Mrad.

The samples were arranged around the radiation source so as to receive the required radiation dose uniformly in 2 hours. Thus the dose rate varied between 0.025—0.40 Mrad/hour according to place of exposure. The absorbed radiation doses were assessed by chemical dosimetry, using the copper sulfate-complemented variant of Fricke's ferrosulfate solution (HART & WALSH, 1954).

1.3. Storage experiments

The samples were stored at 0—4 °C. At each dose level 40—60 samples were treated. The samples were checked at regular intervals during the storage period and the deteriorated cuts were removed. The results of the preservation experiment will be published in detail elsewhere (KISS et al., 1970), this report deals exclusively with the radiation sensitivity of the packaging material.

1.4. Examination of the polypropylene foil

Spectrophotometric and mechanical strength examinations were performed on the polypropylene pouches recovered from the spoiled samples and washed and cleaned carefully for the tests.

1.4.1. Infrared spectrophotometry. The structural study of high polymers often necessitates the assessment of the degree of crystallinity, to distingish for instance isotactic and atactic polymers or, to put it more precisely, to establish the proportion of crystalline to amorphous structural regions.

Cleaned foil cut into appropriate pieces was used for the spectrophotometric measurements. A UNICAM-type SP 200 recording infrared spectrophotometer was used at 1/2 E energy level and at 23 °C temperature applying air as reference. Infrared spectra taken each in 5 replicates at 810, 974, 995, 1215, 1300, 1330, 1360, 2550, 2700, 3150, 4225 and 4300 cm^{-1}, respectively, were interpreted.

1.4.2. Ultraviolet spectrophotometry. The measurements were performed in a PERKIN—ELMER-Type 137 visible and ultraviolet light spectrophotometer. The extinction values measured at 230, 250, 270, 290, 310, 330, 350, 370 and 390 nm as a function of the radiation dose, were compared.

1.4.3. Tensile strength and elongation. At every dose level, foil samples of 100×10 mm, cut either in machine or transverse direction, 10 each, were tested for tensile strength and elongation at rupture, using a "VEB Werkstoff Prüfmaschinen" (Leipzig) tensile strength tester, at a rate of 90 mm/min. elongation, and 50 mm clamping distance. Measurements were carried out at room temperature and 60% relative humidity. (Tensile strength is the force exerted on unit cross section (cm^2 at the moment of rupture.) The elongation at rupture is the elongation of the test specimen (mm) at the moment of rupture as related to its initial length, per cent.)

1.4.4. Mathematical-statistical evaluation of the results. The experimental data were examined for meeting Dixon's r criterion and those meeting it were evaluated by variance analysis and Bartlett's test.

2. Results and discussion

2.1. Evaluation of data obtained by infra-red spectrophotometry

The effect of ionizing radiation on the structure of the polypropylene foil was assessed by comparison of the transmission values measured at different wavelengths of the infra-red absorption spectrum (SCHNELL, 1954).

Different absorption values were measured at the following wavelengths: 810, 974, 995, 1215, 1300, 1330, 1360, 2550, 2700, 3150, 4225 and 4300 cm^{-1}. The transmission values measured at 810, 995, 1300 and 1330 cm^{-1} were

Table 1

Effect of ionizing radiation on the infra-red spectrum of "Moplefan BT" type, thickness 0.005 cm

Comparison of the transmission measured at some chracteristic wavelengths of the infrared spectrum with a "Unicam SP. 200" spectrophotometer

Radiation dose (krad)	Transmission % of infrared light ($\bar{x} \pm s$) at wavelengths (cm^{-1})					
	810	974	995	1215	1300	1330
0	65.4 ±1.68	23.4 ±0.68	31.8 ±0.55	83.2 ±3.61	59.8 ±0.44	66.0 ±0.27
50	66.0 ±1.88	24.5 ±0.97	32.8 ±0.62	76.9 ±2.30	60.9 ±0.99	64.6 ±1.37
100	66.3 ±0.64	24.9 ±1.03	33.6 ±0.92	80.8 ±3.39	60.8 ±0.65	66.5 ±0.87
200	67.2 ±2.12	24.4 ±1.81	33.1 ±2.33	82.0 ±3.93	61.5 ±1.37	66.0 ±1.69
400	67.0 ±1.44	25.1 ±1.94	34.1 ±2.41	84.5 ±3.86	62.0 ±2.01	65.9 ±1.25
800	67.6 ±1.22	25.5 ±1.53	34.1 ±1.54	82.5 ±4.09	62.5 ±1.46	67.1 ±1.31

Radiation dose (krad)	Transmission % of infrared light ($\bar{x} \pm s$) at wavelengths (cm^{-1})					
	1360	2550	2700	3150	4225	4300
0	23.1 ±0.39	79.2 ±1.57	67.2 ±0.58	80.8 ±0.97	78.1 ±1.02	77.3 ±0.32
50	23.7 ±0.79	76.4 ±1.92	66.6 ±1.62	78.8 ±1.59	76.2 ±1.33	75.3 ±1.39
100	24.0 ±0.81	80.2 ±2.16	67.9 ±0.95	80.5 ±0.63	78.0 ±1.16	77.2 ±0.76
200	23.8 ±2.02	81.5 ±3.73	67.8 ±0.99	82.3 ±3.11	78.8 ±1.76	77.7 ±1.40
400	24.5 ±2.13	84.2 ±3.50	68.2 ±1.30	82.6 ±3.03	76.9 ±1.62	77.8 ±1.35
800	25.3 ±1.94	82.0 ±3.41	68.6 ±1.45	82.8 ±3.21	80.1 ±2.88	78.0 ±1.45

$\bar{x}$ = mean of 5 measurements; s = standard deviation

Figures underlined by a solid line indicate significant increase of transmission at a given wavelength upon irradiation.

Figures underlined by broken line indicate transmission values significantly lower in the treated than in the untreated samples.

characteristic of the degree of crystallinity — isotacticity — in the polymer, whereas the transmission at 974 cm^{-1} of the extent of atacticity.

Mean values and standard deviations deduced from the above measurements are summarized in Table 1.

Table 2

Bartlett's test for homogeneity of deviations in Table 1

Total degrees of freedom	Σ DF	= 288
Variance	s_e^2	= 3.5768
	c	= 1.08449
	$x^2_{calc.}$	= 139.4968
	$x^2_{5\%}$	= 328.29
	$x^2_{calc.}$	$\leq \times^2_{5\%}$

There is no significant difference between the deviations

Table 3

Results of variance analysis of the data shown in Table 1

Source of variance	Sum of squares	Degrees of freedom	Variance	F
Total	161,196.87	359		
Wavenumber	160,201.17	11	14,563.74	10,646.00***
Radiation dose	340.28	5	68.06	49.74***
Interaction	261.53	55	4.76	3.47***
Replicates	393.90	288	1.37	

$P^{***} \leq 0.1\%$

Least significant difference between means of any two combinations:

$$SD_{5\%} = 1.379$$

On testing the standard deviations for homogeneity by Bartlett's test (Table 2) no significant difference was observed ($P = 5\%$).

Analysis of variance of the data in Table 1 showed irradiation to significantly modify infra-red absorption in the polypropylene foil (Table 3).

The transmission values marked by underlining in Table 1 are those which differed from the original transmission of the foil at a higher level than the least significant difference ($P = 5\%$) at the given wavelength, as assessed by significance tests following upon the analysis of variance.

2.2. Evaluation of data obtained by UV absorption spectrophotometry

There being no preferential wavelengths in the ultraviolet absorption spectrum, extinction values were read at 230, 250, 270, 290 nm i.e. at 20 nm intervals and averages calculated from 5 replicates were evaluated. The related standard deviations of the mean are shown in Table 4.

The data of Table 4 were examined by Bartlett's test for inhomogeneity of the standard deviations (Table 5) and by analysis of variance for the eventual influence of high energy radiation on the UV absorption spectrum of the foil. The results of variance analysis are shown in Table 6.

Table 4

Effect of ionizing radiation on ultraviolet light absorption of "Moplefan BT" type polypropylene foil of 0.005 cm thickness

Comparison of extinction values as measured in 5 replicates at certain wavelengths of UV spectra taken with a Perkin-Elmer UV 137 spectrophotometer

Radiation dose (krad)	$E_{1\,cm}$ ($\bar{x} \pm s$) at wavelengths (nm)								
	230	250	270	290	310	330	350	370	390
0	1.260	0.987	0.788	0.670	0.545	0.485	0.468	0.453	0.441
	±0.120	±0.083	±0.069	±0.062	±0.054	±0.028	±0.050	±0.049	±0.048
400	1.260	0.986	0.802	0.668	0.504	0.475	0.452	0.432	0.417
	±0.012	±0.013	±0.018	±0.018	±0.061	±0.015	±0.014	±0.013	±0.016
800	1.361	1.068	0.885	0.758	0.611	0.512	0.526	0.509	0.493
	±0.030	±0.016	±0.013	±0.021	±0.018	±0.014	±0.016	±0.017	±0.018

Figures underlined indicate extinction values significantly higher ($P \leq 5\%$) in treated than in untreated samples at the same wavelength

$\bar{x}$ = mean of 5 measurements

s = standard deviation

Table 5

Bartlett's test for homogeneity of deviations shown in Table 4

Total degrees of freedom	DF	= 135
Variance	s_e^2	= 0.001826
	c	= 1.0864
	$x^2_{calc.}$	= 98.489
	$x^2_{5\%}$	= 162.9
	$x^2_{calc.}$	$\leq x^2_{5\%}$

The deviations do not differ significantly

The value of the least significant difference was determined by significance test and on this basis the extinctions differing significantly ($P = 5\%$) for irradiated and non-irradiated foil at the same wavelength were marked in Table 4. Obviously, the polypropylene foil exposed to 800 krad showed significantly greater UV absorption than the untreated foil at most wavelengths examined. This change is, however, practically irrelevant, being only about 10% (Table 7).

Table 6

Results of variance analysis of the data in Table 4

Source of variance	Sum of squares	Degrees of freedom	Variance	F
Total	10.838354	134		
Wavelength	10.456091	8	1.3070	709.8***
Dose	0.167552	2	0.08377	45.5***
Interaction	0.015861	16	0.0009913	0.538***
Replicates	0.19885	108	0.0018412	

$P*** = 0.1\%$

Least significant difference between means of any two combinations:

$$SD_{5\%} = 0.053$$

Table 7

Wavelength (nm)	Increase of UV light absorption (extinction) of the polypropylene foil upon irradiation with 800 krad %
230	7.9*
250	8.2*
270	11.2*
290	11.3*
310	11.2*
330	5.5
350	11.2*
370	11.2*
390	11.2

* = Statistically significant difference ($P \leq 5\%$)

2.3. Effect of ionizing radiation on the tensile strength and elongation at rupture of the polypropylene foil

Results of the measurements of tensile strength are shown in Table 8.

Since only few data were available on specimens cut in transverse direction, statistical evaluation was carried out only on data obtained in machine direction. Prior to statistical analysis, the data were checked by Dixon's r criterion (SVÁB, 1967) to exclude extreme values marked with broken line in Table 8.

The main data and results of variance analysis are shown in Table 9.

The data of Table 9 show that up to 600 krad dose level, there was no significant difference between the tensile strengths of the irradiated and non-irradiated foils, whereas dose levels of 700—800 krad produced a statistically

Table 8

Effect of ionizing radiation on the tensile strength of "Moplefan BT" type polypropylene foil of 0.005 cm thickness

Testing of 100 × 10 mm specimens of polypropylene foil by means of a VEB Werkstoff-Prüfmaschinen (Leipzig) tensile strength tester at a rate of 90 mm/min. elongation and at 50 mm clamping distance. Measurements were carried out at room temperature and 60% relative humidity

Tensile strength (kp/cm²)									
0		400		600		700		800	
krad									
G	H	G	H	G	H	G	H	G	H
295	183	275	240	208	—	240	—	225	275
320	263	270	208	224	—	310	—	285	—
320	267	274	288	232	—	230	—	225	—
265	273	326	240	248	—	270	—	230	—
325	333	177	240	328	—	280	—	205	—
270	227	290	—	288	—	240	—	205	—
320	—	260	—	304	—	224	—	220	—
260	—	270	—	344	—	272	—	215	—
320	—	270	—	288	—	227	—	280	—
380	—	—	—	—	—	200	—	—	—
260	—	—	—	—	—	—	—	—	—

G = machine direction
H = transverse direction
— = not tested
The figures underlined by broken line in the column for 400 krad were found to be extreme in calculations for *Dixon's r*-criterion.

highly significant difference, about 20% decrease of the tensile strength as measured in machine direction.

Results of the elongation tests and their statistical evaluation are shown in Tables 10 and 11.

The data suggest that irradiation already at 400 krad effected a notable increase of the elongation of the polypropylene foil.

Table 9

Variance analysis of tensile strength data in machine direction in Table 8

Irradiation dose (krad)	n	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$ (%)
0	11	303.2	11.23	100
400	7	272.7	3.43	90.2
600	9	273.8	15.99	90.3
700	10	249.3	10.37	82.3
800	9	232.2	9.93	76.7

$x^2 = 12.60$, as calculated by Bartlett's test
$x^2_{1\%} = 13.3$ (DF $= k-1 = 4$)
The deviations do not differ significantly

Results of the F-test

Factor	SQ	DF	MQ	F
Total	78,605.24	$n-1 = 45$		
Inter-group difference	29,058.96	$k-1 = 4$	7264.74	6.01* ($F_{5\%} = 5{,}71$)
Intra-group difference	49,546.28	$n-k = 41$	1208.45	

The mean values differed significantly

Results of the t-test

Comparison of mean values for irradiated and unirradiated (0 krad) foils

Irradiation dose (krad)	t
400	1.81
600	1.88
700	3.56**
800	4.54***

where * $P \leq 5\%$
** $P \leq 1\%$
*** $P \leq 0.1\%$

Table 10

Effect of ionizing radiation on the elongation at rupture of "Moplefan BT" type polypropylene foil of 0.005 cm thickness

The conditions of measurement were the same as indicated in Table 8

Elongation at rupture (%)									
0		400		600		700		800	
krad									
G	H	G	H	G	H	G	H	G	H
266	216	209	274	251	—	404	—	188	229
238	208	211	299	278	—	472	—	219	—
240	260	219	381	267	—	350	—	203	—
212	279	248	274	246	—	436	—	192	—
222	307	364	258	387	—	420	—	186	—
195	308	340	—	304	—	331	—	185	—
215	—	332	—	348	—	387	—	218	—
167	—	346	—	394	—	322	—	214	—
200	—	374	—	352	—	237	—	213	—
243	—	—	—	—	—	212	—	—	—
175	—	—	—	—	—	—	—	—	—

G = machine direction
H = transverse direction
— = not tested
There were no extreme values as assessed by calculations for *Dixon*'s *r*-criterion

Table 11

Analysis of variance of machine direction data of the elongation at rupture in Table 10

Irradiation dose (krad)	n	$\bar{x}$	$s_{\bar{x}}$
0	11	215.7	9.10
400	9	293.7	23.40
600	9	314.1	19.19
700	10	357.1	26.65
800	9	202.0	4.79

$x^2 = 25.11$ as calculated by Bartlett's test
$x^2_{0,1\%} = 18.5$ (FG $= k-1 = 4$)
Differences between the standard deviations are statistically highly significant.
According to the results of the chi-square test the deviation at 700 krad dose level is significantly higher, whereas that at 800 krad significantly lower than the rest.

Results of the F-test, disregarding the data at 100 and 800 krad dose levels

Factor	SQ	DF	MQ	F
Total	130,013.17	$n-1 = 28$		
Inter-group differences	54,947.17	$k-1 = 2$	27,473.59	9.52***
Intra-group differences	75,032.88	$n-k = 26$	2,885.88	($F_{5\%} = 3.37$)

Difference between the mean values is highly significant

Results of the t-test

Comparison of mean values for irradiated and unirradiated (0 krad) foils

Irradiation dose (krad)	t
400	3.10**
600	4.07***

where ** $P \leq 1\%$
*** $P \leq 0.1\%$

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EFFECT OF PIMARICIN, ASCORBIC ACID AND GRAPE JUICE PROTEINS ON RADIATION TOLERANCE OF WINE YEASTS IN GRAPE JUICE

I. FÁBRI, K. VAS and G. STEHLIK

(Received August 12, 1970)

The effects of various additives and food constituents on the radiation resistance of yeasts were studied in grape juice as model solution to obtain information for the elaboration of radiation preservation methods for food products.

The highly radioresistant yeast strain *Saccharomyces cerevisiae* var. *ellipsoideus* was used as the test organism.

Addition of ascorbic acid to food products has been advantageous not only in respect of increasing the vitamin content, but also with regard to a possible decrease in the "radiation-taste" of irradiated fruit juices. There is reason to suppose that vitamin C exerts a certain protective effect not only on the aroma components but also on the yeast cells. In the present experiments a vitamin C concentration of 2500 ppm had no influence on the death rate of yeasts when irradiated with 100 or 200 krad either at room temperature or at 55 °C. Heat treatment at 55 °C for almost 20 minutes and simultaneous irradiation with 200 krad reduced the yeast count of the grape juice by at least 6 orders of magnitude.

The above combination treatment seems to ensure a satisfactory preservation of fruit juices with low (10^{2-3}/ml) yeast content. The applied mild heat treatment and radiation treatment are individually ineffective.

The polyene antibiotic pimaricin was, at a level of 20 ppm, fungicidal for grape juices with 10^6/ml cell count, whereas at levels of 10 ppm or below it exerted a fungistatic effect only.

Addition of 20 ppm pimaricin and subsequent irradiation with 500 krad was entirely ineffective against yeast cell growth in grape juice. This suggests the decomposition of the anti-fungal antibiotic by irradiation. However, post-irradiation treatment with 10 ppm pimaricin within one hour after exposure to 500 krad was sufficient to inhibit yeast growth in grape juice over a 3-month period of storage at 28 °C.

The effect of grape proteins on the radiation sensitivity of yeast cells was examined immediately upon irradiation and after storage in the irradiated medium for 1 week at 5 °C. There was, apparently, no immediate protective effect, but a certain protection was observed during storage, probably due to the binding by grape proteins of the decomposition products affecting the metabolism of the yeast cell.

The main problem of the radiation preservation of certain fruit juices is the usually considerable alteration of sensory qualities caused by the radiation. FERNANDEZ, STEHLIK & KAINDL (1966) showed the radiation dose, required to ensure microbiological stability of grape juices to be 800—1800 krad, depending on the initial cell count, while already 200—500 krad are sufficient to produce a notable change of taste.

Investigations into the radiation preservation of fruit juices have been conducted along two main lines, one being the protection against radiation

damage of the components responsible for flavour, odour and colour, the other the study of factors influencing the radiation sensitivity of the microflora, primarily of yeasts, responsible for spoilage, with the aim of reducing their sterilization requirement.

In an attempt to reduce the dose of radiation, ionizing radiations have been combined with other agents of preservative effect, *e.g.* chemicals or heat treatment. These agents are supposed to alter the metabolism of the living cells so as to sensitize them to lower doses of radiation.

Ascorbic acid added to grape juice for preservation of the aroma was examined for its radioprotective effect on yeast cells and the anti-fungal antibiotic pimaricin was tested for its value in reducing the radiation dose requirement. Preliminary investigations were carried out also into the effect of the protein content of grape juice on the radiation resistance of yeast cells. The data obtained in these experiments may be helpful in establishing radiation preservation technologies also for food products other than grape juice.

Addition of ascorbic acid to grape juice increases not only the latter's nutritional value and vitamin content, but also diminishes the radiation-induced changes of taste. Irradiation does not reduce the total quantity of ascorbic acid, it produces only a shift in the ascorbic acid — dehydro-ascorbic acid ratio (BALLA & KISZEL, 1960). There is reason to suppose that, by its double bond, ascorbic acid binds part of the free radicals formed under the influence of irradiation, reducing thereby the radiolysis of aroma and colour substances and that of proteins as well. The decomposition products of the proteins play an important role in the formation of the so-called "radiation-taste" (KOVÁCS, 1970).

This hypothesis, nevertheless, postulates the removal of free radicals from the system, thereby diminishing the effect of radiation on yeasts. To obtain more information on this problem, several series of experiments were carried out in which grape juices enriched with vitamin C were irradiated either at room temperature or with simultaneous heat treatment at 55 °C. There is experimental evidence (FARKAS et al., 1963; ILLI, 1966) that the combination of a mild heat treatment at 55 °C and irradiation considerably increased the yeast-destroying effect without altering the sensory qualities of fruit juices.

The antibiotic pimaricin (Myprozin) (American Cyanamid Co., Pearl River, New York) is a product of *Streptomyces natalensis*. As a polyene-macrolide antibiotic, it is active against many yeast and mould species (PATRICK et al., 1958; RYHAGE, 1963). It has been used primarily for the extension of the storage life of fruit juices and concentrates (SHIRK & CLARK, 1962, 1966).

The mechanism of action of this antibiotic has been hypothetically explained by its binding to the compounds of sterane structure in the cell wall of fungi and thereby changing its semipermeability (DEMELL & DEENEN, 1965). The advantages of pimaricin lie in its activity over a wide range of pH,

and its resistance to boiling, however, it is sensitive to ultraviolet irradiation (YORK, 1966).

The advantage of pimaricin over sorbic acid, widely used in Hungary, is that it is effective in concentrations 10 to 50 times lower and does not affect taste. Experiments were, therefore, performed to establish whether the radurization dose may be reduced by the use of pimaricin and if so, the simultaneous or the consecutive application of the two agents (radiation treatment and pimaricin) seems more advantageous.

It has been established that the decrease of the logarithms of cell count, as a function of increasing radiation doses, is non-linear and the survival curve, at least in its initial phase, is sigmoid in nature (ILLI, 1966). This circumstance suggests among others also an influence of the actual grape juice components on the radiation sensitivity of yeasts. The protective role of proteins in relation to heat treatment has been known for long. According to analyses by RADOLA (1966), the protein content of grape juices varies between 0.01 and 0.001 %, which is a fairly small concentration. The protective value of proteins in the grape juice was examined in samples with protein contents increased tenfold, immediately upon irradiation and during storage in the irradiated medium.

According to the literature, the storage in irradiated medium increases the efficiency of radiation treatment and of other antimicrobial agents through the action of degradation products on the radiation-damaged microbial cells.

1. Materials and methods

1.1. Test organism

A radiation-resistant *Saccharomyces cerevisiae* var. *ellipsoideus*, isolated by FERNANDEZ and STEHLIK (1966) from the juice of *Grüner Veltliner* grapes, was used throughout. The LD_{50} of the yeast strain was 85 krad at room temperature (KISS, 1969).

1.2. Nutrient medium

The yeast strain was cultivated in grape juice as nutrient medium. Freshly pressed grape juice was placed in PVC pouches and frozen and stored at −28 °C. The thawed samples were passed through a Seitz filter and diluted 1 : 1 with tap-water.

The pH of the grape juice was adjusted to 5.0 by the addition of NaOH, 2 % agar-agar was added and the medium was sterilized at 115 °C for 30 minutes. The liquid agar-containing medium of about 80 °C was distributed automatically, under aseptic conditions, into Petri dishes, 10 ml into each. The plates were stored for 2 to 3 days to allow condensed water to evaporate.

1.3. Irradiation medium

The original medium was grape juice. Frozen grape juice was thawed, passed through a Seitz filter and subsequently through a Millipore filter of 0.45 μm pore diameter, for sterilization. For irradiation, small (3 ml) and larger (20 ml) amounts of the juice were distributed into appropriate test tubes, 120 mm by 15 mm (diameter) and 160 mm by 82 mm (diameter), respectively, under aseptic conditions, in three replicates.

In experiments on grape juice proteins, an 0.14 M (25.5 g/litre) mannitol solution was also used as irradiation medium.

The test material was enriched with grape proteins isolated from Veltliner grape juice by means of precipitation with ammonium sulfate. The precipitate was centrifuged and dissolved in 0.01 M phosphate buffer (pH 7.2). By appropriate purification, a 9 % protein solution was obtained to which 0.1 % merthiolate was added for preservation (RADOLA et al., 1967). The preservative was then removed by dialysis against 0.1 M phosphate buffer for 2 days at 2—4 °C. The protein solution was added to the grape juice at a concentration level of 0.1 % and the enriched material was sterilized by passing it through a Millipore filter.

1.4. Inoculation of the irradiation medium

One or two loopfuls of the yeast strain cultivated on grape juice agar slants at 28 °C for 24 hours were transferred into 5 ml portions of sterile grape juice. The inoculum thus prepared was homogenized in a Whirlmix apparatus. According to ILLI (1966), the yeast culture takes 24 hours to reach the exponential phase of growth in which all but a few cells are in an identical physiological state. Aliquots of the inoculum were then pipetted into the irradiation medium. The initial yeast cell count was adjusted to 10^6—10^7 /ml and checked by means of a Buerker cell counting chamber. Prior to irradiation, the inoculated grape juice was incubated overnight at 5 °C, to allow the cells to adapt to the new medium.

1.5. Radiation treatment

The tubes were placed in appropriate racks for exposure. A Gamma Cell 220 (Atomic Energy of Canada) was used as the radiation source. The dose rate at the place of exposure was previously checked by means of Fricke's ferrous sulfate method. In the experiments, a 100 krad treatment was given within 7 minutes (dose rate: about 850 krad/hr). Radiation treatment at 55 °C was performed in the special container developed for the purpose by ILLI and STEHLIK. The jacketed container was filled with water and when the appropriate temperature was reached, the tube rack was placed into the container. The

temperature of the water jacket was adjusted by means of an ultrathermostat placed outside the radiation source and connected with the container by rubber tubing. Since water absorbs part of the ionizing radiation, the time of exposure at 55 °C had to be extended to 9 min. 40 sec. in order to reach a radiation dose corresponding to 100 krad.

1.6. Determination of the number of cells surviving radiation

The cell count was determined by streak plate technique. Within 1 hour after irradiation a (1 + 9) dilution series was prepared from the radiation-treated suspension in sterile distilled water. At each dilution level three streak plates were prepared. 0.25 ml of the suspension was streaked onto the surface of the agar-agar medium by means of a thin glass rod with triangular end. The plates were incubated for 7 days at 28 °C and afterwards the colonies were counted. The reason for extending the usual culturing time was that, considering the retardation of yeast growth by irradiation, the usual 3—4 days were found insufficient for the outgrowth of colonies. The average yeast cell counts and the standard deviations were assessed from results read on 3—9 plates. Yeast survival curves were plotted as a function of the radiation dose.

2. Results

2.1. Combination treatment: ascorbic acid and irradiation

2.1.1. Effect of ascorbic acid on radiation tolerance of yeasts in grape juice at room temperature. 3-ml samples of grape juice inoculated with yeast (8.78×10^6/ml) and enriched with 2500 ppm ascorbic acid were irradiated at dose levels of 100 and 200 krad. The results are shown in Fig. 1.

As can be seen from Fig. 1, the presence of 2500 ppm ascorbic acid in the grape juice had no influence on the radiation tolerance of yeasts at 100 or 200 krad.

2.1.2. Effect of ascorbic acid on the radiation resistance of yeasts irradiated at 55 °C. 3 ml samples of grape juice inoculated with yeast (1.56×10^7/ml) and enriched with 2500 ppm ascorbic acid were irradiated with 100 and 200 krad, resp., as described under paragraph 1.6. The survival curve is shown in Fig. 2.

As can be seen from Fig. 2, heat treatment at 55 °C for almost 20 minutes had, in itself, no notable influence on yeast destruction, whereas in combination with irradiation at dose levels of 100 and 200 krad it decreased the initial cell count by 2 and at least 6 orders of magnitude, respectively. The presence of 2500 ppm ascorbic acid caused no change in the radiation tolerance at 55 °C.

2.2. Effect of the combined use of pimaricin and irradiation on the inhibition of yeast growth in grape juice

2.2.1. Growth inhibitory action of pimaricin on yeast strains. Prior to the study of the combined effect of irradiation and pimaricin it had to be clarified what kind of inhibitory effect pimaricin alone would exert on yeast growth

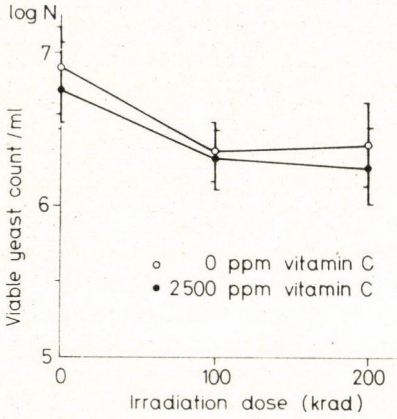

Fig. 1. Effect of addition of ascorbic acid on the radiation tolerance of wine yeast in grape juice irradiated at two different dose levels at room temperature. The vertical lines represent the standard deviation ($\pm$ s)

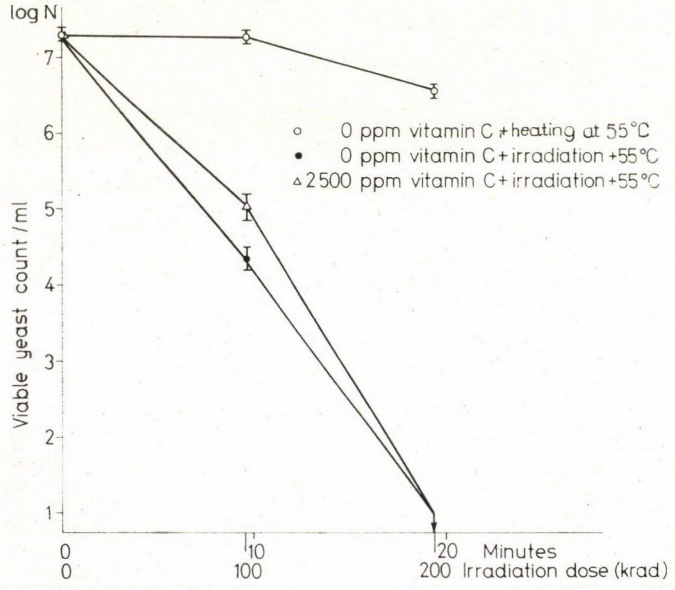

Fig. 2. Effect of addition of ascorbic acid on the radiation tolerance of yeast in grape juice, heat-treated at 55 °C and irradiated, simultaneously. The vertical lines represent the standard deviation ($\pm$ s)

in grape juice. For this purpose, pimaricin was added at concentrations of 2.5, 5, 10 and 20 ppm to 50 ml grape juice samples containing yeast cells at a level of 5.57×10^6/ml. The samples were stored at 28 °C for 30 days. Yeast cell counts were checked several times during incubation. The results are summarized in Table 1.

The data of Table 1 permitted of the following conclusions:

1. Pimaricin at a 20 ppm level had a fungicidal effect.
2. Pimaricin at a 10 ppm level had a fungistatic effect.

Table 1

Growth inhibitory action of various concentrations of pimaricin on yeast cells in grape juice after storage for 30 days at room temperature

Concentration of pimaricin (ppm)	Viable cell count/ml as a function of storage time (days)				
0	5.57×10^6	fermented	fermented	fermented	fermented
2.5	5.57×10^6	4.82×10^3	fermented	fermented	fermented
5.0	5.57×10^6	9.96×10^3	4.22×10^4	fermented	fermented
10.0	5.57×10^6	1.93×10^3	2.66×10^3	3.3×10^4	fermented
20.0	5.57×10^6	2.0×10^0	0	0	0

In the latter case an initial decrease of the yeast cell count was observed, thereafter the surviving cells started to grow and in due course the grape juice underwent fermentation. The fungistatic effect of 2.5 ppm pimaricin, the lowest concentration used, lasted for at least 6 days.

2.2.2. Joint effect of pimaricin and radiation on the survival of yeast cells. In the next series of experiments, the effect of exposure to 250—500 krad within one hour after the addition of pimaricin at 10 or 20 ppm levels was tested on viable yeast cells (Fig. 3).

Fig. 3 clearly shows that pimaricin did not increase the cell-destructive action of radiation. When stored, all the samples, including those initially containing 20 ppm pimaricin, underwent, within 2—3 days, fermentation, suggesting thereby the decomposition of the antibiotic upon irradiation.

2.2.3. Effect of post-irradiation treatment with pimaricin on the survival of yeast cells in grape juice. 5-ml samples of grape juice containing yeast cells at a concentration of 10^5/ml were irradiated with 250 or 500 krad and within one hour various concentrations of pimaricin (5, 10, 20 ppm) were added, under aseptic conditions. The samples thus treated were stored for 2 months at 20 °C and the results were evaluated by assessing the number of tubes in which fermentation had taken place. Each radiation dose and each concentration of pimaricin was examined in 4 replicates (Table 2).

As can be seen in Table 2, post-irradiation treatment with 10 ppm pimaricin of the samples irradiated at 500 krad dose level ensured satisfactory preservation. Pimaricin concentrations less than 10 ppm had no notable effect to this end.

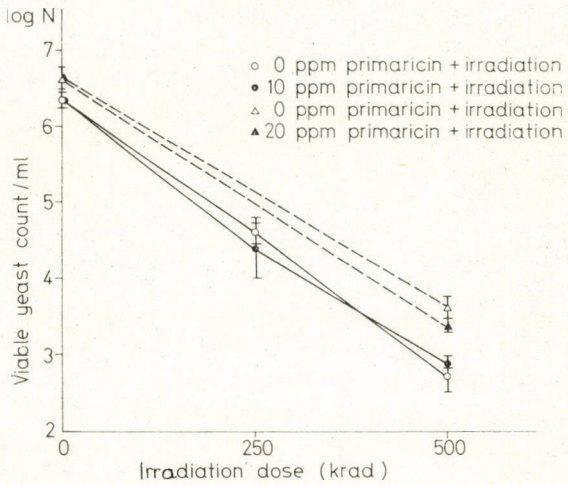

Fig. 3. Combined effect of two different doses of pimaricin plus irradiation on survival of yeast cells in grape juice. The vertical lines represent the standard deviation ($\pm$ s)

Table 2

Effect of post-irradiation treatment with pimaricin on the storage life of grape juice of 10^5/ml initial cell count, as assessed after storage for 60 days at 28 °C

Number of fermented samples of 4 replicates, 5 ml each, at each dose level

Radiation dose (krad)	Concentration of pimaricin (ppm)			
	0	5	10	20
0	4	4	4	0
250	4	4	4	0
500	4	4	0	0

2.3. Effect of grape proteins on the radiation tolerance of yeasts

Grape juice samples enriched with ten times the normal concentration of grape proteins (see paragraph 1.4) were adjusted to a yeast cell count of 3×10^6/ml and samples of 3 ml were irradiated with 250 or 500 krad. Normal grape juice exposed to the same radiation doses served as the control. Viable yeast counts were checked immediately upon irradiation (Fig. 4).

The results of the sensory evaluation are in good agreement with those of THORNLEY (1957) and COLEBY et al., (1960).

Fig. 4 shows the yeast survival curves in grape juice samples with normal and increased (0.1%) grape protein content, resp., to be identical when tested immediately upon irradiation. Apparently, the protein content of the grape juice had no direct influence on the radiation-treated yeast cells.

To check the eventual protective effect of grape proteins on yeast cells stored in irradiated medium, an 0.14 M mannitol solution containing 0.1% grape protein was inoculated with the yeast strain, then irradiated with 200—250 krad, stored for 1 week at 5 °C and finally examined for viable yeast count (Fig. 5).

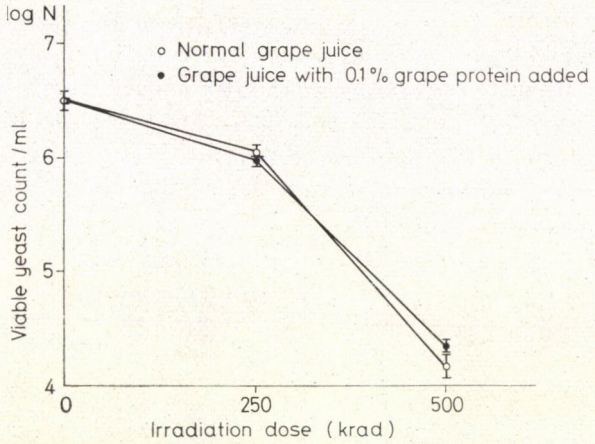

Fig. 4. The effect of enrichment with grape proteins on the survival of yeast cells in grape juices irradiated at two different dose levels. The vertical lines represent the standard deviation (± s)

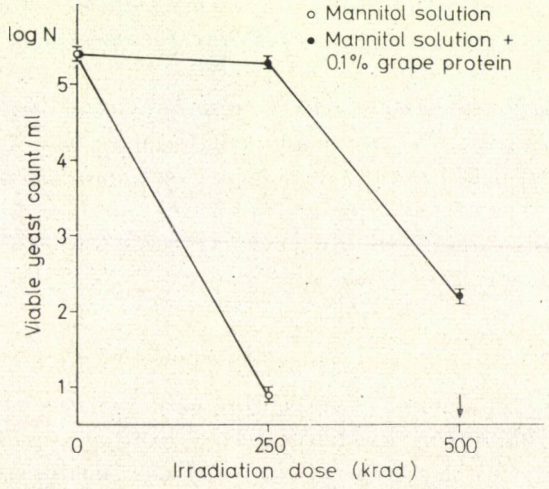

Fig. 5. Survival curve of yeast cells in a mannitol solution (0.14 M) containing 0.1% grape protein after post-irradiation storage for 1 week at 5 °C. The vertical lines represent the standard deviation (± s)

As can be seen in Fig. 5, the presence of protein in the mannitol solution had a considerable protective effect on the yeast cells as compared with those stored in plain mannitol solution without added protein.

3. Conclusions

3.1. Ascorbic acid

The presence of 2500 ppm ascorbic acid in the grape juice medium had no influence on the radiation sensitivity of yeast cells either at room temperature (22 °C) or at 55 °C. This implies that ascorbic acid does not protect yeasts against radiation. Similar conclusions were drawn by EMBORG (1966) from studies on moulds during the same period. He found vitamin C treatment not to influence the radiation resistance of the particularly thermoresistant mould species *Byssochlamys fulva* in grape juice either at room temperature or at 55 °C.

Consistently with the findings of FARKAS, VAS and KISS (1963) as well as ILLI (1966), the combination of heat treatment at 55 °C and irradiation with 200 krad resulted in considerable destruction of yeast cells.

This kind of combined treatment will perhaps resolve the problem of the microbiological stabilization of low-yeast fruit juices without notable alteration of taste.

3.2. Pimaricin

At a concentration of 20 ppm, pimaricin was in itself sufficient for the preservation of grape juice with a yeast content of 10^6/ml. Exposure at 250 krad apparently decomposes the anti-fungal antibiotic, whence simultaneous treatment with pimaricin and radiation seems pointless. Similar observations were made by DRAXLER (1966) in experiments with sorbic acid and sodium benzoate.

Post-irradiation treatment with 10 ppm pimaricin of grape juice exposed to 500 krad appeared sufficient to prevent fermentation. It should be noted in this context that post-irradiation chemical treatment has as yet been difficult on a plant scale. The effect of pimaricin on the radiation-damaged yeast cells being very intensive, relatively low concentrations were sufficient to stabilize the radiation-treated grape juice.

3.3. Grape proteins

The protein components of grape juice had obviously no direct protective effect on yeast cells during irradiation. They exerted, nevertheless, a certain protective action during storage in the irradiated medium, perhaps through inactivation of the decomposition products of radiolysis, inhibitory to living cell metabolism.

The experimental work was carried out during the tenure of an IAEA Fellowship held by the first author at the Seibersdorf Reactor Centre (Österreichische Studiengesellschaft für Atomenergie), in 1966.

*

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RHEOLOGICAL INVESTIGATIONS ON LIQUID WHOLE EGGS

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Various liquid whole egg preparations were tested for viscosity, their rheograms were taken and regression calculations were performed on the results.

Liquid whole egg homogenized to a minor degree corresponded rheologically to a Bingham body and could be characterized by the appropriate equation.

Exposure to shear stress effected structural disintegration of the egg, with corresponding reduction of structural viscosity and transformation from gel into sol state, so that the egg substance corresponded almost or completely to a Newtonian liquid. On a plant scale, this state can be readily determined by Engler's viscosimeter, particularly if the homogenized egg material has been filtered as well.

In the case of hen's eggs the energy required to bring 1 ton egg material into the above specified liquid state has been estimated as 0.5—0.7 kWh.

Some decades ago the main users of fresh eggs were the households. But in the meantime, with the expansion of institutional catering and the extending range of ready-to-cook and finished food products and pastes, the industrial use of eggs and the container transport of liquid whole eggs have increasingly gained ground. This has initiated closer investigations into the rheological properties of the liquid whole egg, with the aim to improve transport conditions and preserving technologies.

The anatomy and chemical composition of the egg, which were described in great detail by KÖNIG (1903), are not discussed here. Mention is made only of the fact that the freshly broken egg corresponds to a colloidal dispersoid system with known macroscopic, microscopic and submicroscopic structure. These characteristics notably influence its rheological properties.

The rheological properties of the liquid whole egg have been little studied, owing perhaps to the great variability of the egg as a biological material. Variability rests, with the breed of the layer (ARROYAVE et al., 1957), and with the age of the layer (ARROYAVE et al., 1959). ARROYAVE and his coworkers have considered also additional influences of diet, conditions of management, etc. on egg quality. As a viable matter, the egg itself changes with time, by its vital and enzymic functions. YOSHINOBU NONAMI (1954) described decrease of viscosity with the ageing of the egg. The decrease of viscosity of the liquid egg on sonication, as reported by POPOV & DOGANOVA-KOLEVA (1958), may have been related to the destruction of the the submicroscopic structure.

The rheological examination of the egg presents several problems. The macroscopic structural elements (e.g. chalaza, various membranes, etc.) clog the capillary pipette and cause jamming of the rotating viscometers, while the microscopic and submicroscopic structural elements impart a gel-like elastic consistency to the freshly broken egg, preventing its flow through the capillary pipette.

A further difficulty of the rheological study of egg is the alteration of its substance by the instruments in operation. Particularly the rotation viscometers, but also the instruments operated by the principle of capillary flow, break up or destroy the microscopic and submicroscopic structure of the liquid whole egg. This implies not only a structural change of the material during measurement, but a fundamental difference between the original material and that regained after measurement.

In this institute, examinations of the liquid whole egg have been carried out as part of the research programme on materials handling. Closer investigations into the rheological properties of liquid whole egg were aimed at the determination of the parameters relevant to materials handling.

1. Materials and methods

1.1. Materials

1.1.1. Preparation of liquid whole egg samples. First class commercial eggs were broken and homogenized individually in glass beaker by breaking the vitelline sac and stirring yolk and albumen with an electrically operated glass rod stirrer at about 100 rpm for 3 minutes.

1.1.2. Formol-treated liquid whole egg. Samples of commercial liquid whole egg preparations with $2^0/_{00}$ formol added for preservation, according to the adopted technology, were procured from various plants of the National Poultry Processing Trust. In the Budafok and Pesterzsébet Plants Cs-50 screw pumps,* in other plants household washing machines (Hajdúsági Iparművek) have been used for egg homogenization.

1.2. Methods

1.2.1. Measurement of viscosity. Two methods, each based on a different working principle, were used to clarify whether or not Engler's viscosimetry method could be substituted for the expensive, sensitive and complicated rheo-viscosimetry of Epprecht, for the purpose of in-plant measurement of viscosity in liquid whole egg preparations.

Measurements with Engler's viscosimeter were carried out as set forth in the standard prescription MSZ 14724.

* Produced by the firm Fémmechanikai KTSZ, Budapest.

Measurements with Epprecht's Rheomat-15 rheoviscosimeter were carried out in the O-Ring System, using the formula

$$\eta = \text{scale reading} \times \text{stage factor (cP)} \tag{1}$$

where η is the viscosity in cP.

1.2.2. Recording of rheograms was carried out with Epprecht's rheoviscosimeter, using the O-Ring System. First the shear stress was determined:

$$\tau = \text{scale reading} \times \text{tabulated factor} \tag{2}$$

where τ is the shear stress, dyne/cm^2.
Subsequently, shear stress was plotted as a function of shear velocity:

$$D = f(\tau) \tag{3}$$

where D is the shear velocity sec^{-1} and τ is the shear stress, dyne/cm^2.

Also equations of regression were calculated for the curves thus obtained.

Note

Epprecht's Rheomat-15 rheoviscosimeter is an instrument operated on the principle of rotation. Of the two coaxial cylinders the movements of the internal cylinder are controllable by a ratio switch in a manner according to which high stages provide higher, low stages lower shear velocities at a given slit size. Measurements are, as a rule, started by increasing the speed and this series is designated as "a" or speed increasing experiment. The speed is increased until there is a readable displacement on the instrument scale which shows the degree of the damping effect of the liquid. Subsequently, the speed is reduced, this being the "b" or speed reducing experiment. Measurements were made at intervals of 2 minutes. The scale was read at each velocity rate and the value of the corresponding shear stress, viz. viscosity was calculated on the basis of the table supplied with the instrument.

2. Results

2.1. Viscosity of whole liquid egg samples

Measurements with Engler's viscosimeter could be carried out exclusively on the formol preserved liquid whole egg samples procured from the plants in Budafok and Pesterzsébet. Mean values of serial measurements on 10 replicates each, are shown in Table 1.

As can be seen in Table 1, lowest viscosity was measured in samples originating from the spray-drying plants. In these plants, the liquid is filtered

after homogenization and filtration removes the macroscopic structural parts (chalaza, membranes, etc.).

Freshly broken eggs do not flow down in Engler's viscosimeter; coarsely homogenized liquid whole egg preparations or those containing structural elements clog the viscosimeter. The same phenomenon was noted also with the formol treated samples homogenized in household washing machine.

Table 1

Viscosity of formol-preserved liquid whole egg preparation expressed in Engler degrees

(Each figure represents the mean of 10 measurements)

Place of origin	Temperature °C	E°
Spray-drying plant in Pesterzsébet	10	2.12
	20	2.12
	30	1.29
Budafok plant	10	4.80
	20	4.50
	30	4.13

Table 2

Viscosity in cP of individual liquid whole egg samples measured at 20 °C and low gear

Serial number	Mode of measurement	
	a^x	b^+
1	8.89	7.01
2	8.38	6.50
3	10.59	9.23
4	14.02	11.62
5	13.33	8.21
6	12.31	7.18
7	17.43	11.11
8	13.85	10.25
9	10.60	6.83
10	14.53	11.45
Mean values	12.40	8.94
Standard deviation	±2.79	±2.05

x = speed increasing series
+ = speed reducing series

The same applies also to Epprecht's instrument with the difference that here inhomogeneity of the test material brings about jamming of the rotating part. This renders the reading of the scale difficult and thereby reduces the accuracy of the measurement.

Table 3

Viscosity in cP of formol-preserved liquid whole egg samples measured at low gear at different temperatures

(Mean values)

Temperature °C	Origin of samples									
	Budafok plant		Pesterzsébet plant		Sárvár plant		Győr plant		Törökszentmiklós plant	
	a[x]	b[+]	a	b	a	b	a	b	a	b
15	7.67	6.83	—	—	43.7	41.9	41.0	36.9	32.6	28.2
20	6.95	6.72	20.67	17.21	42.6	40.0	34.3	32.3	26.4	26.2
25	5.52	5.21	20.52	17.20	38.3	34.5	30.8	27.8	21.7	18.3

[x]"a" = speed increasing series
[+]"b" = speed reducing series

Viscosity values measured in the homogenate of single eggs with Epprecht's rheoviscosimeter are shown in Table 2, while those measured in formol-preserved samples originating from various plants in Table 3.

Each value represents the mean of 10 measurements. The Tables clearly show that viscosity values measured in the speed increasing series were always higher than those measured in the speed reducing series.

2.2. Rheograms of liquid whole egg samples

For the recording of rheograms, the shear stress had to be determined first.

Shear stresses measured at 20 °C in samples from fresh eggs and in formol-preserved liquid whole egg samples are shown in Tables 4 and 5, respectively.

The data of Table 5 have been presented graphically as rheograms in Figs 1 to 4.

As can be seen from the Figures, samples from the Budafok and Pesterzsébet spray-drying plants produced straight lines, chracterized by

$$y = mx \tag{4}$$

whereas the rheograms of the other samples are characterized by

$$y = mx + b \tag{5}$$

Table 4

τ values measured in individual liquid whole egg samples at 20 °C

Gear	Shear stress, dyne/m²											
	1		2		3		4		5		6	
	a	b	a	b	a	b	a	b	a	b	a	b
1	2.47	1.95	2.33	1.80	2.95	1.73	3.90	3.23	3.04	2.28	3.42	2.00
2	3.90	2.85	3.33	2.52	4.19	2.54	5.23	4.52	4.28	3.23	4.38	2.90
3	5.23	3.85	4.28	3.57	5.62	3.61	7.14	6.09	5.25	4.42	5.62	4.09
4	6.81	5.33	5.81	4.85	8.57	4.85	9.52	8.33	7.14	6.14	7.14	5.52
5	8.95	7.13	7.71	8.95	9.90	6.66	10.14	11.05	9.52	8.19	9.28	7.52
6	12.86	10.62	11.19	9.52	14.38	8.58	18.09	16.09	13.33	11.95	10.62	10.92
7	17.14	14.38	14.76	12.76	19.33	13.05	24.29	21.81	17.86	16.28	17.19	14.67
8	22.38	19.05	19.05	16.67	25.48	17.43	30.95	28.67	22.86	21.57	19.29	19.29
9	29.38	25.72	24.86	22.14	37.43	23.33	40.00	38.81	27.62	29.14	28.10	25.81
10	37.15	34.76	31.91	29.19	43.58	36.77	—	—	41.43	40.00	35.72	34.53

a = speed increasing series
b = speed reducing series

Table 5

τ values of formol-preserved liquid whole egg samples at 20 °C

Gear	Shear stress, dyne/m²									
	Budafok plant		Pesterzsébet plant		Sárvár plant		Győr plant		Törökszentmiklós plant	
	a	b	a	b	a	b	a	b	a	b
1	1.67	1.67	7.14	5.24	11.91	11.43	7.62	6.67	9.53	9.05
2	2.38	2.14	9.05	7.14	14.74	14.77	10.00	8.57	12.86	11.91
3	3.33	3.10	11.91	9.29	20.00	19.05	12.38	11.43	16.67	17.15
4	4.52	4.29	15.24	11.91	26.20	25.24	15.72	14.29	21.91	20.00
5	6.19	6.09	18.10	15.24	34.74	31.91	20.00	18.58	27.63	25.72
6	9.29	8.57	23.33	20.35	45.72	44.77	57.32	26.20	38.10	34.72
7	12.38	13.81	29.53	26.96	—	—	35.82	33.10	45.49	42.20
8	16.19	15.00	33.81	33.04	—	—	—	—	—	—
9	21.43	20.00	45.72	43.82	—	—	—	—	—	—
10	27.62	29.96	—	—	—	—	—	—	—	—
11	38.10	37.62	—	—	—	—	—	—	—	—

a = speed increasing series
b = speed reducing series

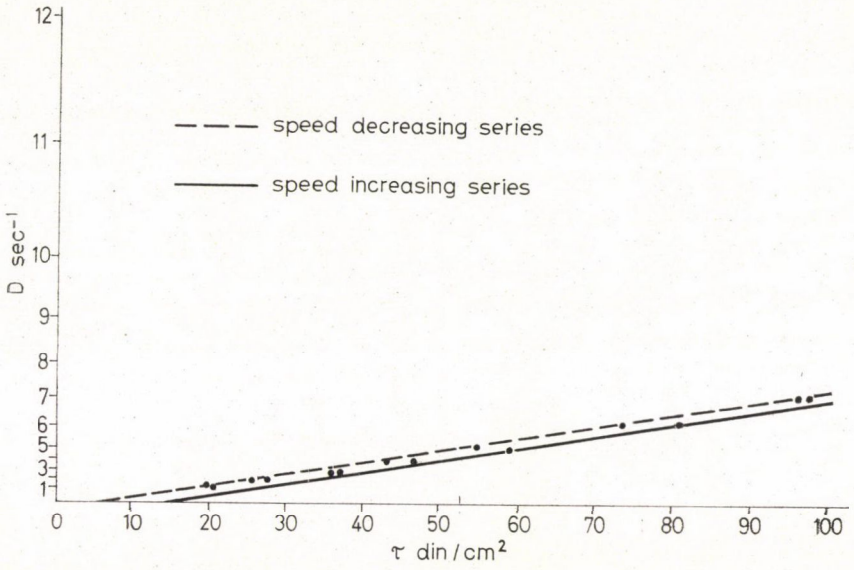

Fig. 1. Rheogram at 20 °C of a liquid egg sample from the Törökszentmiklós plant

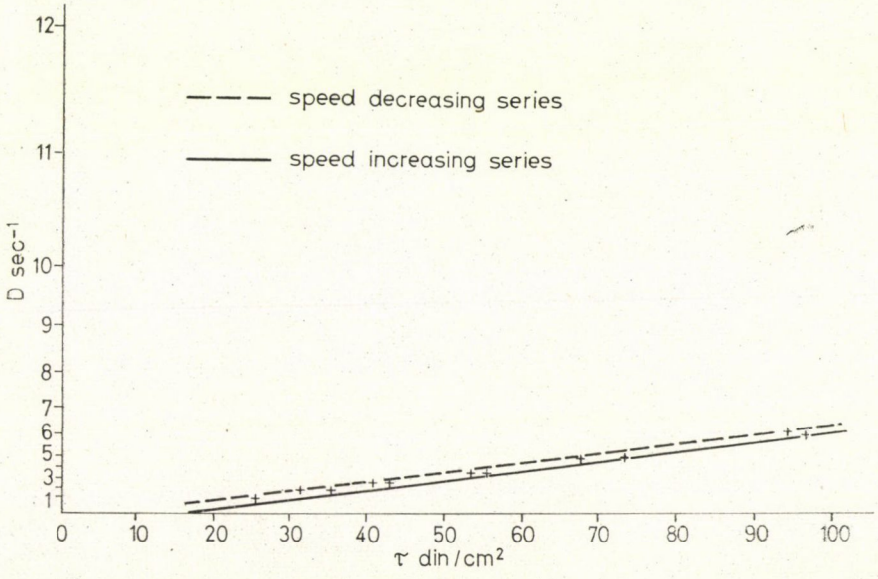

Fig. 2. Rheogram at 20 °C of a liquid egg sample from the Sárvár plant

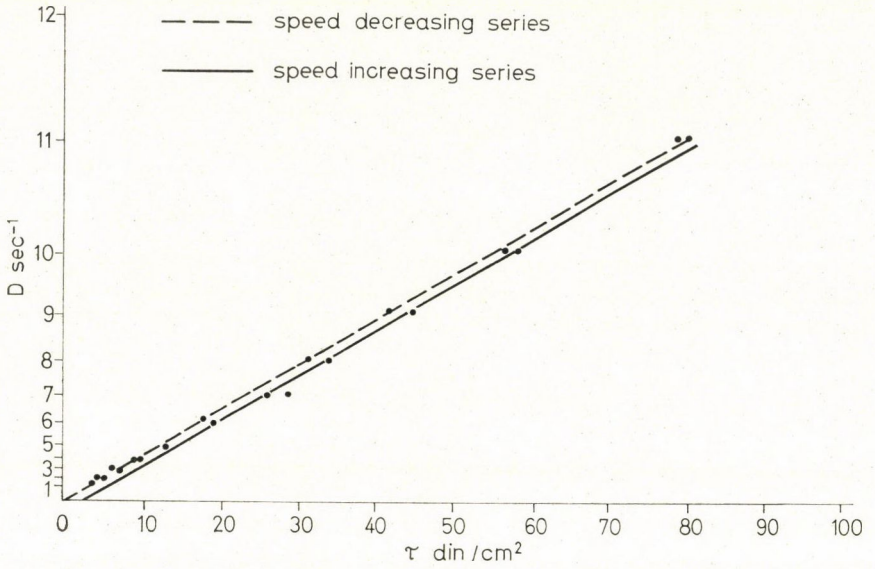

Fig. 3. Rheogram at 20 °C of a liquid egg sample from the Budafok plant

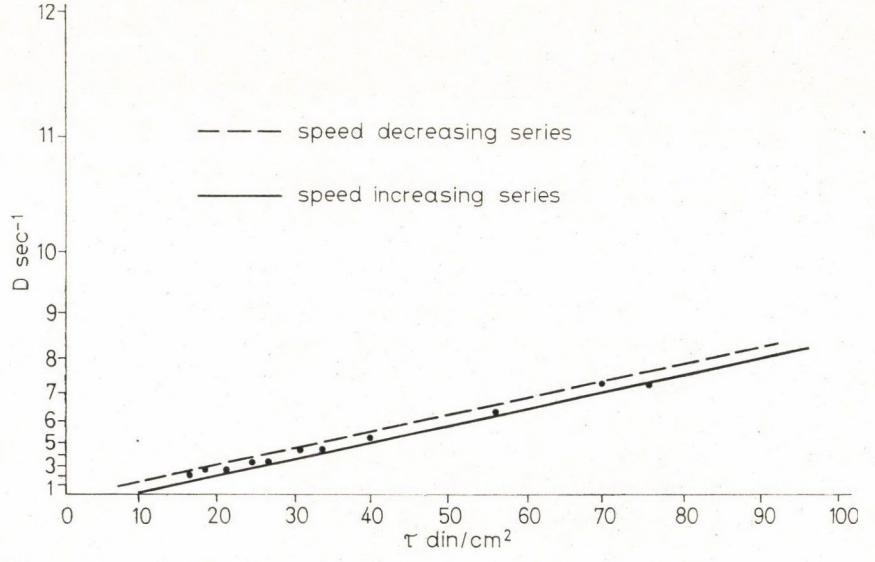

Fig. 4. Rheogram at 20 °C of a liquid egg sample from the Győr plant

Calculating the regressions for the rheograms, the following values were obtained for the formol-preserved samples:

Sample from the Budafok plant	$y = 0.969x$
Sample from Pesterzsébet spray-drying plant	$y = 0.829x - 0.86$
Sample from Sárvár plant	$y = 0.865x - 4.13$
Sample from Győr plant	$y = 1.07x - 8.1$
Sample from Törökszentmiklós plant	$y = 1.11x - 2.0$

Table 6

Calculations of regression for rheograms of individual liquid whole egg samples

Temperature	Experiment	Equation of regression	Standard deviation of	
			m-value	*b*-value
10 °C	a	$y = 0.855x - 0.49$	± 0.41	± 0.17
	b	$y = 0.896x - 0.30$	± 0.238	± 0.254
20 °C	a	$y = 0.878x - 5.7$	± 0.321	± 0.86
	b	$y = 0.778x - 2.2$	± 0.024	± 1.27
30 °C	a	$y = 1.590x - 8.2$	± 0.316	± 1.60
	b	$y = 1.380x - 5.6$	± 0.366	± 1.13

Regressions were calculated also for the homogenates of individual eggs examined serially. In the average, 60 measurements were performed at each temperature level and the results were calculated at 95% probability level. The results are summarized in Table 6.

Both the rheograms and calculations of regression show the lines derived from series of "b" measurements to have steeper slopes as compared to those derived from the series of "a" measurements.

2.3. *Virtual energy requirement of egg homogenization*

In the spray-drying plants, liquid whole egg is prepared by homogenization with a Cs-50 screw pump, which implies transformation of a Bingham body-type substance to an approximately Newtonian liquid.

The capacity of the Cs-50 pump is 5000 litre/hour for water and 2000—2500 litre/hour for eggs. Considering the 2.2 kW power output of the driving motor, it is obvious that only about half of the above capacity is utilized for the transportation of the liquid, the other half being used up for homogenization. It follows that the specific energy requirement of homogenization is about 0.5—0.7 kWh/ton.

3. Conclusions

As established in the foregoing, the rheograms of liquid whole egg preparations homogenized in a household washing machine can be characterized by the function

$$y = mx + b \tag{5}$$

which, in rheological terms is

$$D = \frac{\tau}{\eta} + f_B \tag{6}$$

where τ is the shear stress, dyne/cm^2, D the shear velocity, sec^{-1}, f_B the yield stress of Bingham bodies and η the viscosity, cP.

Materials producing such rheograms can be regarded as Bingham bodies which behave like elastic materials up to the yield stress, f_B, and behave like plastic materials above the yield stress.

The rheograms of liquid whole egg preparations homogenized with screw pump followed the function

$$y = mx \tag{4}$$

or, in terms of rheology

$$D = \frac{\tau}{\eta} \tag{7}$$

and thus they can be regarded as Newtonian liquids.

The rheograms also indicate that samples exposed to the mechanical effects of the speed increasing series "a" behaved rather like Newtonian liquids during the speed reducing series "b", as shown by the steeper slopes of the curves and drop of the f_B values. This suggests that liquid whole egg, which originally had the nature of a Bingham body tended to approximate the characteristics of a Newtonian liquid during mechanical processing to become like one at the end of processing.

As assessed from the transport capacity of the screw pump, the energy used up for the Newtonian transformation of one ton of liquid whole egg corresponded to 0.5—0.7 kWh specific work input.

Considering that in the rheograms

$$\eta = \operatorname{ctg} \alpha \tag{8}$$

where η is the viscosity and α the angle enclosed by the straight line, and considering that the input mechanical work effected a falling tendency of η, it seems obvious that the viscosity of the egg can be reduced by the destruction of its micro-structure. In other words, the submicroscopic structure of the natural egg imparts to it a gel-like elasticity which, under the influence of

mechanical work, assumes first the characteristics of a Bingham body and with additional input shear stress those of a Newtonian liquid; simultaneously, its colloidal nature transforms to that of a sol and its viscosity approximates a limit value.

The viscosity, which exceeds the above limit value and depends on the submicroscopic structure of the liquid whole egg and with the elimination of that structure, theoretically should fall to zero, can be considered the structural viscosity. For a given liquid whole egg preparation the structural viscosity can be terminated by a well defineable shear stress. In the practice, this energy requirement proved to be about 0.5—0.7 kWh/ton, the input of which renders the liquid egg water-like, with a viscosity of only a few *cP*.

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A THIN LAYER CHROMATOGRAPHIC METHOD TO DETERMINE THE PIGMENT CONTENT (COMPONENTS) IN THE PERICARP OF PAPRIKA

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A new method has been elaborated for the determination of the pigment content, or the individual components of the colour substances in the pericarp of paprika by thin layer chromatography.

The pigment components present in the fruit as fatty acid esters are released by saponification with 5% alcoholic KOH; to prevent the decomposition of the pigment components by the alkali, KOH is added at a level corresponding to the pigment content of the sample according to Benedek.

Saponification is allowed to take place in a water bath of 45 °C for 60 minutes. Subsequently, the ether phase is washed with distilled water to remove the alkali, dried with Na_2SO_4 and evaporated in nitrogen flow over a water bath of 45 °C. The pigment adhering to the wall of the container is dissolved in 5 ml benzene, and developed twice in a 8 cm wide band on a Kieselgel G layer of 0.25 mm, previously activated for 4 hours at 130 °C, using petrolether-benzene-glacial acetic acid-acetone (40 : 10 : 2.5 : 2.5) as a developing solvent. After evaporation of the solvent, the individual components are eluted with methanol, made up to the required volume and their extinctions are read by spectrophotometry.

The identification and quantitative determination of the pigment components are performed by means of calibration curves of standard preparations.

The most conspicuous property of paprika is its colour which varies in all shades from dark to bright red. A bright red colour is one important requirement of marketability. This initiated closer investigations into the nature of the colouring components of paprika and the elaboration of a method for their identification, with the aim to determine which of the main components is responsible for changes in the colour of paprika. The elucidation of these aspects is expected to make possible the protection of the colour components and the prevention of colour changes.

The paprika pigment consists mainly of carotenoids. The latter are polyene pigments built from isoprenes; they are alicyclic compounds and their chromophore system contains many double bonds which predispose for cis-trans-isomerism taking place in the central part of the molecule. According to recent investigations, most carotenoids are in the trans configuration. Their colour may be of any shade from yellow to deep red.

KARRER (1948) and GOODWIN (1952, 1955) divided the carotenoids into two groups:

a. The carotenes are unsaturated hydrocarbons which dissolve readily in petrolether and less readily in alcohol;

b. the xantophylls are oxygen containing carotenoid derivatives (alcohols, aldehydes, esters) which dissolve readily in alcohol and less readily in petrol-ether.

The paprika contains beta-carotene, but its main colour components are xanthophylls. The most important xantophyll compounds are
of the polyene alcohols:

cryptoxanthin,
zeaxanthin,
lutein,

of the polyketones:

capsantin
capsorubin

In paprika, the polyene alcohols and polyene ketones are present in the form of esters; as esters of fatty acids they can be in fact regarded as pigment waxes.

Investigations into the colouring matter of paprika were pioneered by ZECHMEISTER and CHOLNOKY (1937), who identified the red colour components as capsanthin and capsorubin and the yellow components as beta-carotene, cryptoxanthin and zeaxanthin. CHOLNOKY et al. (1937) elaborated an adsorption chromatography method for the isolation of these components and isolated more than 20 xantophylls and determined their structure and quantity. Expressing the pigment content in capsanthin, it varies as a rule between 3 and 8 g for 1 kg paprika solids. The percentual proportions of the main components are

capsanthin	52—60%
capsorubin	10—18%
beta-carotene	8—13%
zeaxanthin	8—10%
lutein	8—10%
cryptoxanthin	3— 5%

As these compounds constitute the bulk of the colouring matter of paprika, they are dealt with in more detail in this study.

In structural studies of epoxycarotenoids, KARRER and JUCKER (1950) demonstrated that most unknown components of the paprika colour belong to this group.

Later CHOLNOKY et al. (1958) refined their original column chromatography method as follows: The colour components of paprika are extracted with ether and saponified with 20% alcoholic KOH. The alkali is then removed by washing and the ether by distillation; the dry residue is dissolved in 1 : 1 petrolether-methanol solvent and applied to a column of calcium carbonate + calcium hydroxide. The fractions separated on the column are eluted in

methanol, filtered, shaken in benzine and the traces of methanol are removed by washing with water. Both the red and yellow components show a yellow colour on dissolution in benzine, they are identified by spectrophotometry at 465 nm.

Both the original and the improved method of CHOLNOKY et al. (1937, 1958) being time consuming, there has been an increasing demand for a simple and rapid routine procedure which would enable the determination of the total pigment content of paprika. BENEDEK (1958) elaborated a technique by means of which the total pigment content could be determined in grammes of capsanthin for 1 kg dry matter. The absorption maximum of capsanthin in benzene is at 488 nm, the molecular extinction of capsorubin is about 10% higher, while that of carotene, cryptoxanthin and zeaxanthin are about 5% lower; considering that the latter three components make about twice the amount of capsorubin, this difference becomes equalized and the colour of the benzene extract is measured at 492 nm and expressed as capsanthin. BENEDEK (1958) observed that if the yellow colouring components are predominant, the conversion of the total pigment content to capsanthin gives a lower value than the actual pigment content.

Benedek's method has been very helpful in the breeding and qualification of paprika, as it enables a rapid assessment of the total pigment content by an index number which, however, does not inform on the qualitative and quantitative relationships of the individual components.

1. Materials and methods

1.1. Materials

Pure zeaxanthin, capsanthin and capsorubin supplied from the Institute of Chemistry, Medical University, Pécs, and beta-carotene, lutein and standard cryptoxanthin from the firm Hoffmann LaRoche were used as reference preparations.

1.2. Comparative study of techniques

Our aim was to elaborate a rapid technique which, beyond the determination of the total pigment content would also enable the differentiation of the components. Thin layer chromatography was selected for this purpose.

Thin layer chromatography has been recently widely used for carotenoid analysis; we tested this and some other methods proposed in the literature for that purpose.

Most pigment components of paprika are waxes, that is different fatty acid esters of polyene alcohols. BOLLINGER (1962) observed that the chromato-

graphic behaviour of the carotenoids depends on the position of the double bonds, viz. on the structure of the terminal ring (open or closed), as adsorption is stronger with the open ring. According to STAHL (1967), the carotenes and polyesters being apolar compounds, they run faster in apolar than in polar solvents. Under such conditions the Rf values of the carotenes are higher than those of the oxygen containing compounds. As xanthophyll and polyesters are difficult to separate in a thin layer, attempt was made to determine the various types of polyene alcohols and ketones formed on saponification of the esters. Several authors are of the opinion that the carotenoids are not separable in a single step on a single plate. It was, therefore, attempted in various ways to separate from one another the compounds of different polarities.

Certain authors tried to separate compounds of different polarity by stepwise development on a single plate, using different solvent systems. Following the method of ISLER et al. (1967), we employed a calciumhydroxide-Kieselgel G (6 : 1) layer, using petrolether-benzene solvent system (98 : 2) to separate the hydrocarbon containing carotenoids, benzene to separate those of ketone type and benzene-methanol (98 : 2) to separate those containing a hydroxyl group. But, despite of running the material three times, separation of the above components was unsatisfactory and the carotenoids became damaged by the long procedures of development.

EGGER and VOIGT (1965) reported that polyamide is suitable for the separation of carotenoids, because if a polar solvent is applied, such as a mixture of petrolether + methanol [methylketone (50 : 50) (4 : 1)], the carotene will migrate with the solvent front, while the other polyenes will separate according to their polarities. Using as polar solvent a mixture of water + methanol [methylketone, (50 : 50) (1 : 10)], the sequence of separation will be the reverse, in that the carotene will remain at the start line and the other components occupy positions of different Rf values in the sequence of their polarities. Both procedures were tested in this laboratory, but they did not provide a satisfactory separation.

MONTAG (1962) tried to separate the carotenoids by stepwise development in an activated Kieselgel G layer, using as solvent system chloroform + glacial acetic acid (65 : 2) in the first step and benzene in the second one. Checking of his method in this laboratory revealed that the carotenoids tend to loose colour intensity in the course of repeated development. MONTAG (1962) suggested to stabilize the separated components by spraying with $SbCl_3$ dissolved in chloroform after the solvent has been evaporated; this in fact produced stabilization but it interfered with the quantitative determinations.

Since neither of the above methods produced in itself a satisfactory separation, we tried different methods for carotinoids of different structure. For the carotenes we choose BOLLIGER's method (1964), who separated the alpha-, beta-, and gamma-carotenes by means of a methylenechloride-undekan

(20 : 80) solvent system using Kieselgel-G layer. For polyene alcohols, several methods were available. As the carotenoids decompose rapidly in the dry layer after the solvent has been evaporated, the colour stabilization of the components presented a serious problem. HAGER (1962) proposed impregnation with 5×10^{-3} M ascorbic acid for a layer of mixed Kieselgur G, Kieselgel G, calcium carbonate and calcium hydroxide. He proposed petrol-isopropanol-distilled water (100 : 10 : 1.25) as solvent system. Though this technique yielded reproducible results and impregnation with ascorbic acid stabilized the colour of the carotenoids for 24 hours, it was not suited for our purpose because the beta-carotene migrated with the solvent front and capsanthin and capsorubin were inseparable even by stepwise development.

The partial impregnation technique of EGGER (1962) was also attempted; Kieselgur G coated plate was used and developed with 7% paraffin oil — petrolether solvent system up to 4 cm below the top of the plate. The chromatogram was dried then developed stepwise with methanol-acetone-water (20 : 4 : 3). Thus the separation of the components was sharp and their colour was deepened by the paraffine oil impregnation. Beta-carotene remained at the start line and capsanthin and capsorubin migrated with the solvent front. But paraffin oil rendered a quantitative determination impossible.

BENK et al. (1963) studied the separation of inherent and added carotenoids in orange juices, using a non-activated Kieselgel G layer and petrolether + benzene + acetone + glacial acetic acid (80 : 20 : 2 : 1) as solvent system. This technique did not produce satisfactory results with paprika pigment because with the given solvent, the beta-carotene migrated with the solvent front, while the polyene alcohols did not separate.

We modified BENK's (1963) method using activated Kieselgel G, and increased the polarity of the solvent by the addition of more glacial acetic acid and applied step-technique. Under these conditions, the migration velocity of the apolar compounds could be reduced, while that of the polar compounds could be increased to such extent as to provide an appropriate separation of the paprika pigment components. This modification enabled the determination of all the components in the same layer using a single solvent system.

2. Results

2.1. Elaboration of the chromatographic method

On the basis of the testing of several thin layer chromatographic methods (see paragraph 1.2), we selected for our studies a 0.25 nm layer of Kieselgel G activated for 4 hours at 130 °C and as solvent system we used the mixture of petrolether-benzene-acetone-glacial acetic acid (40 : 10 : 2 : 2.5).

The Rf values of the paprika pigment components were identified by comparison to the values obtained with the carotenoid reference standards. The results are shown in Table 1. Under the applied conditions of chromatography, the reference preparations and the separated paprika pigment components were placed corresponding to their Rf values and polarities.

Table 1

Rf values of pure pigment components and of the corresponding components isolated from paprika
Each value represent the mean of 5 measurements

Pigment components of paprika	Rf values	
	Purified pigment components	Components isolated from paprika
Capsorubin	0.061	0.055
Capsanthin	0.143	0.142
Zeaxanthin	0.350	0.347
Lutein	0.400	0.402
Cryptoxanthin	0.600	0.598
Beta-carotene	0.918	0.915

To establish the purity of the separated pigment components, viz. the success of separation, the fractions of the reference preparations and of the corresponding paprika components were scraped off the plate and examined for absorption spectra at 400—500 nm. Powdered paprika samples of 3.27, 5.67 and 8.20 g/kg pigment contents, expressed in terms of capsanthin according to BENEDEK, were used for this purpose.

The absorption curve for capsanthin was related to 0.1 g powdered paprika/100 ml methanol, while the curves for the other components to 0.5 g powdered paprika/100 ml solvent. The absorption curves for the reference preparations were taken in methanol at a concentration of 0.5 mg/100 ml. The results are shown in the Figures, which permit the following conclusions:

The methanol solutions of capsanthin and capsorubin were red and the absorption curve for capsanthin flattened at the maximum, with both the reference preparation and the fraction separated from paprika. The absorption curve for capsorubin had, like in the benzine solution, one main and two secondary maxima. (Figs 1, 2, 3, and 4).

Absorption curves for beta-carotene and cryptoxanthin in methanol were of similar structure. The two secondary maxima were indistinct in the case of cryptoxanthin even with high pigment concentrations (Figs. 5, 6, 7, 8).

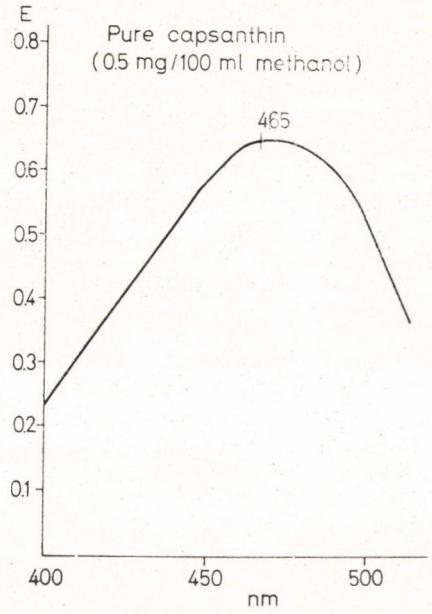

Fig. 1. Absorption curve for methanol-dissolved pure capsanthin, layer: Kieselgel G

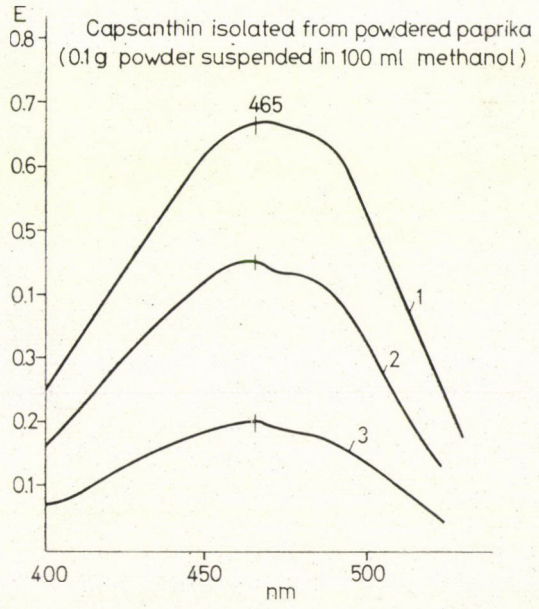

Fig. 2. Absorption curve for methanol-dissolved capsanthin isolated from paprika by chromatography layer: Kieselgel G. The total pigment content, according to Benedek, of the three powdered samples: 8.20 (1), 5.67 (2) and 3.27 (3) g/kg, respectively

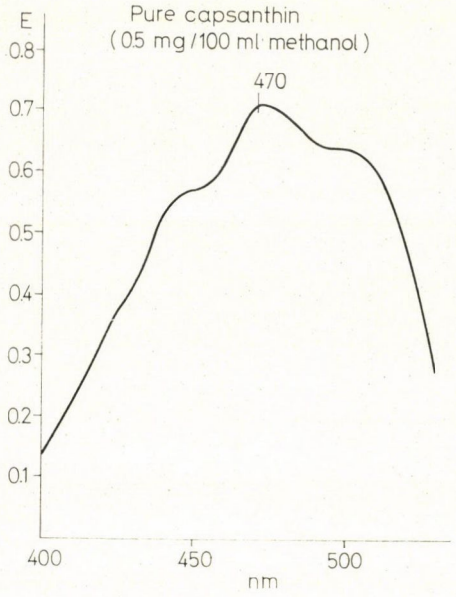

Fig. 3. Absorption curve for methanol-dissolved pure capsorubin, layer: Kieselgel G

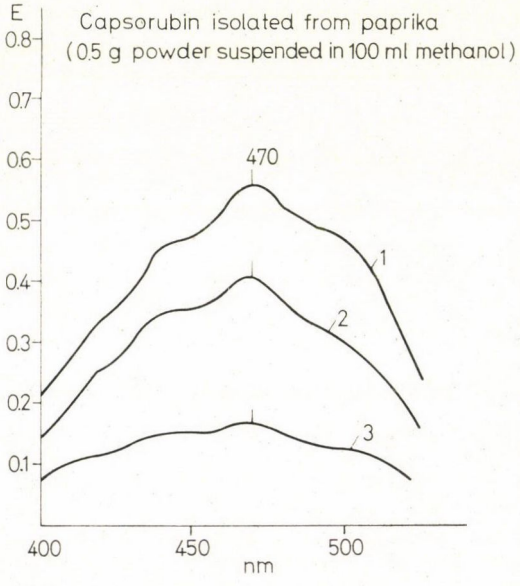

Fig. 4. Absorption curve for methanol-dissolved capsorubin isolated from paprika layer: Kieselgel G. Total pigment content, according to Benedek, of the three powdered samples: 8.20 (1), 5.67 (2) and 3.27 (3) g/kg, respectively

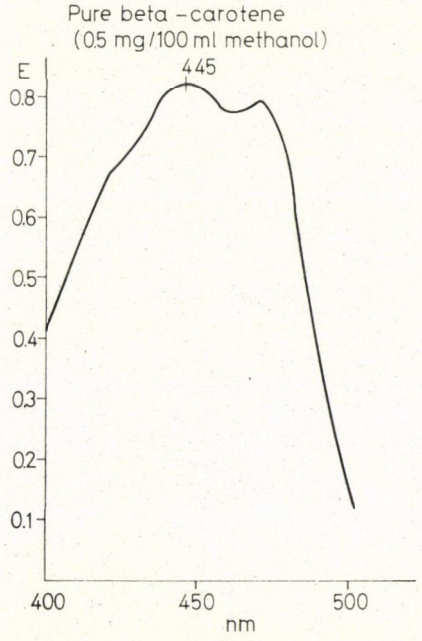

Fig. 5. Absorption curve for methanol-dissolved pure beta-carotene, layer: Kieselgel G

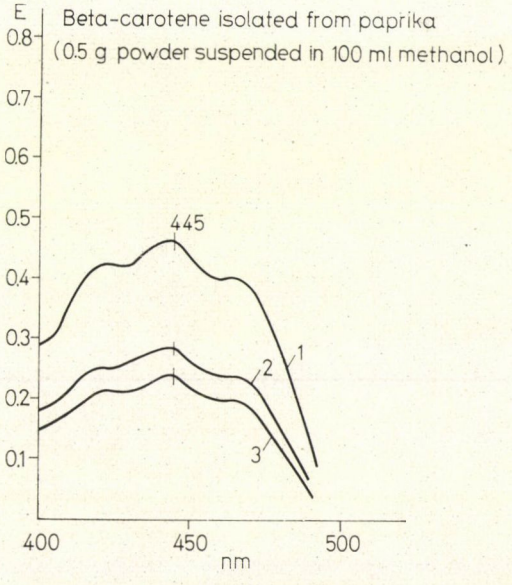

Fig. 6. Absorption curve for methanol-dissolved beta-carotene isolated from paprika by chromatography, layer: Kieselgel G. The total pigment content of the three powdered samples: 8.20 (1), 5.67 (2) and 3.27 (3) g/kg, according to Benedek

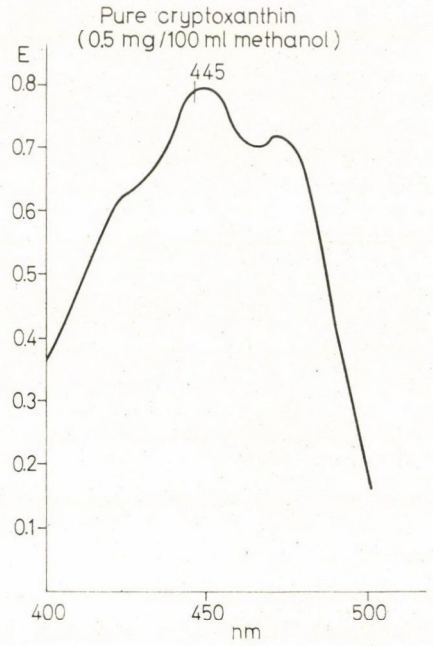

Fig. 7. Absorption curve for methanol-dissolved pure cryptoxanthin, layer: Kieselgel G

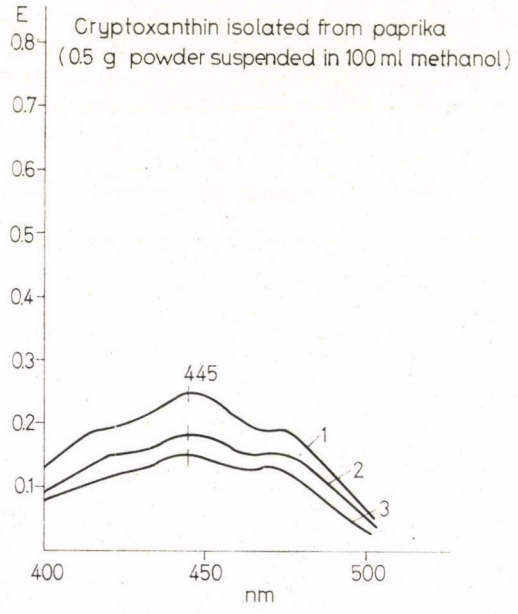

Fig. 8. Absorption curve for methanol-dissolved cryptoxanthin isolated from paprika, layer: Kieselgel G. The total pigment content of the three samples 8.20 (1),5.67 (2),3.27 (3) g/kg, according to Benedek

The methanol solutions of the zeaxanthin and lutein reference preparations showed maxima at 445 and 440 nm, respectively. Both compounds showed one main and two secondary peaks. As these components are present in the paprika at low concentrations, both are polyenedialcohols and their chemical structures and adsorption curves are highly similar, they were eluted together from the developed plate and determined together (Figs 9, 10).

From the Figures and measurements it was inferred that if methanol is used as a solvent, the Rf values and absorption maxima of the reference preparations and the corresponding paprika pigment components are almost identical (Table 2).

Table 2

Absorption maxima (nm) of curves for pure pigment components and for the corresponding components isolated from paprika

Pigment components of paprika	nm of methanol-dissolved					
	pure pigment			isolated components		
Capsanthin	465			465		
Capsorubin	470			470		
Zeaxanthin	445	468		440	465	
Lutein	440	465		440	465	
Cryptoxanthin	425	445	473	420	445	473
Beta-carotene	445	470		420	445	465

For the quantitative determination of the paprika pigment components, the test material was applied in strips (Fig. 11); this technique enables the application of a fairly large quantity of pigment so that even the separated fractions contain sufficient material for elution and measurement. The fractions were eluted in methanol and measured directly for extinction. Methanol-dissolved capsanthin and capsorubin are red-coloured, the other components are yellow-coloured.

The calibration curves for quantitative determination were prepared as follows:

Stock solutions of the pure reference compounds were prepared in benzene (10 mg/10 ml) and quantities of 0.1, 0.15, 0.20 and 0.25 ml were applied 5 replicates each in strips and developed in the solvent system specified above. The separated fractions were eluted in methanol and the extinction values were plotted out graphically. The multiplication factors calculated from each calibration curve are shown in Table 3.

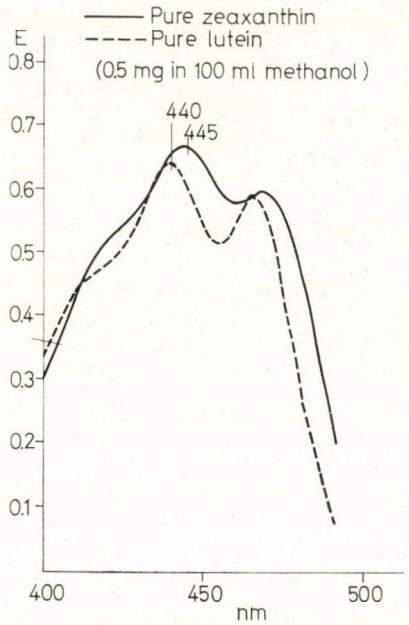

Fig. 9. Absorption curves for methanol-dissolved pure zeaxanthin and lutein, layer: Kieselgel G

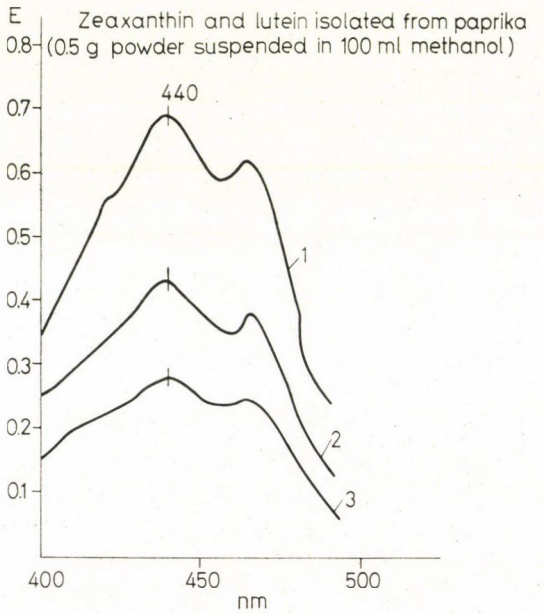

Fig. 10. Absorption curves for methanol-dissolved zeaxanthin and lutein isolated from paprika, layer: Kieselgel G. The total pigment content of the three powdered preparations 8.20 (1), 5.67 (2) and 3.27 (3), according to Benedek

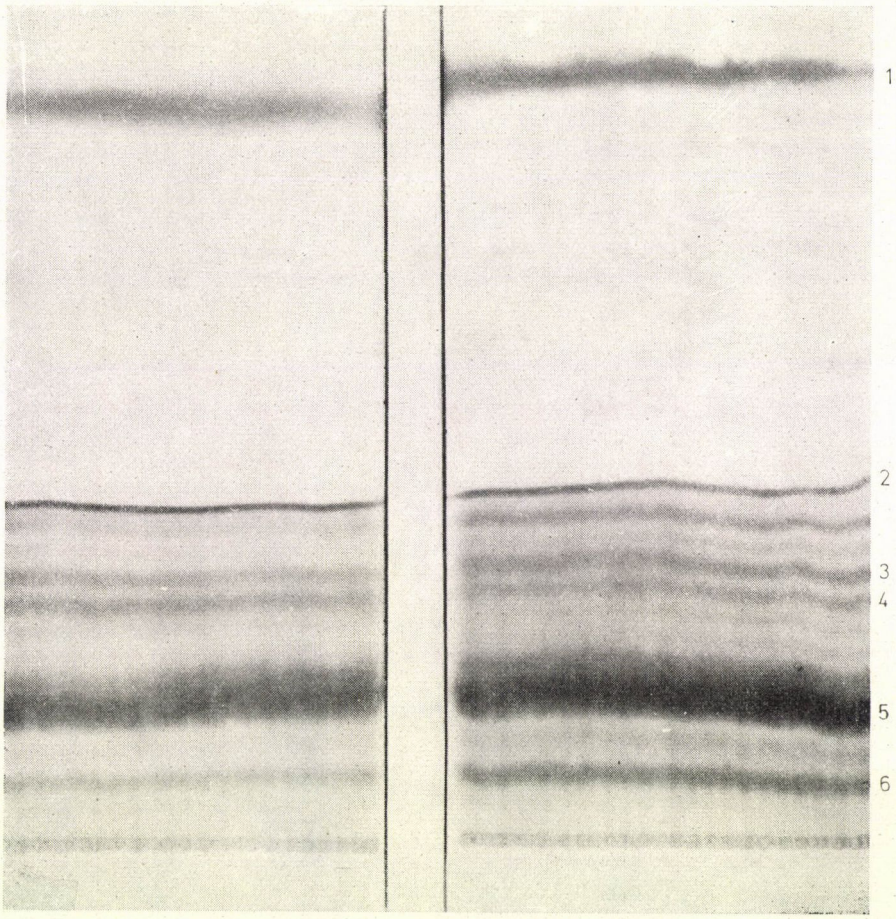

Fig. 11. Separation of the pigment components of paprika by the thin layer chromatography method. Numbers 1—6 mark the positions of the following components. 1. Beta-carotene; 2. Cryptoxanthin; 3. Lutein; 4. Zeaxanthin; 5. Capsanthin; 6. Capsorubin

Table 3

Correction factors calculated from calibration curves for ethanol-dissolved reference preparations

	λ nm	Correction factor
Capsanthin	465	0.700
Capsorubin	470	0.700
Zeaxanthin	445	0.800
Lutein	445	0.800
Cryptoxanthin	445	0.600
Beta-carotene	445	0.600

2.2. Description of the method

Reagents: Petrolether, analytical grade (Reanal, fp. 120)
Benzene, analytical grade (Reanal)
Acetone, analytical grade (Reanal)
Glacial acetic acid, analytical grade (Reanal)
Ether, analytical grade (Reanal)
Methanol, analytical grade (Reanal)
Kieselgel G Merck layers, 20×20 cm, 0.25 mm, activated for 4 hours at 130 °C
KOH, analytical grade (Reanal)

2.3. Procedure

The paprika sample was homogenized and its water content was determined by drying for 4.5 hours at 95° ± 2 °C, according to the standard prescription MSZ 9681—62. The Benedek number was determined in an aliquot part of the sample and 1.0 g of the latter was shaken with 50 ml ether, then 5% alcoholic potassium hydroxide was added in an amount corresponding to the Benedek number, and saponification was allowed to take place for 60 minutes under reflux in a 45 °C water bath. Subsequently the ether phase was washed with distilled water to remove alkali, dehydrated with anhydrous sodium sulfate, filtered and evaporated under vacuum, in nitrogen flow, until dry. The pigment adhering to the wall of the vessel was dissolved in 5 ml benzene and 0.5 ml of it was applied to the chromatography layer in 8 mm wide strips, using a micropipette, and developed in two steps in a solvent system of petrolether-benzene-glacial acetic acid-acetone (40 : 10 : 2.5 : 2), in the dark. The solvent was evaporated and the appropriate spots were scraped off the plate, eluted in methanol, filtered and made up to an appropriate volume for spectrophotometric measurement. The extinctions were measured in a "Spektromom 201" spectrophotometer (MOM, Budapest), fitted with a current adapter, at the maximum wavelengths as established in the absorption curves:

capsanthin	465 nm
capsorubin	470 nm
zeaxanthin, lutein	445 nm
beta carotene, cryptoxanthin	445 nm

In the possession of the correction factors deduced from the calibration curves, the g/kg concentration of the paprika pigment components may be

calculated by the formula

$$\text{g/kg} = \frac{f \times E \times a \times c}{b \times d \times m}$$

where E = the extinction value read in a 1 cm cuvette;
f = the correction factor calculated from the calibration curve for each component;
a = the quantity in ml of the pigment eluted from the chromatogram;
b = the quantity in g of the paprika sample used for saponification;
c = the volume in ml of the stock solution obtained after saponification;
d = the volume in ml of the sample applied to the chromatographic layer;
m = the per cent solids content of the paprika measured after drying at 95 °C.

With the solids content known, the pigment concentration is obtained n g/kg paprika solids. The entire procedure is accomplished in about 4.5 hours.

3. Conclusions

To establish the accuracy of the thin layer chromatography method, five paprika samples of different pigment contents, established according to Benedek, were tested each in five replicates. Standard deviations and the coefficients of variation were calculated for both our and Benedek's procedure. The results shown in Table 4 reveal that the total pigment contents could be determined almost as accurately with the chromatographic method as with Benedek's method, and the coefficient of variation *(v)* was 3.6%.

The total quantity of pigment (in g/kg dry paprika) calculated as the sum of components determined by our method was as a rule by 20—30% higher than that obtained with Benedek's procedure. The quantities of the pigment components in powdered paprika, shown in the rising sequence of their pigment contents are compared to the values obtained by Benedek's method in Fig. 12.

The results show that with increasing total pigment content proportionally increased the red components: capsanthin and capsorubin, and the yellow components: zeaxanthin, lutein, cryptoxanthin and carotene as well.

The percentual distribution of the components in powdered paprika samples of different total pigment contents are shown in Fig. 13. The two red components capsanthin and capsorubin varied between 50—60 and 9—16%, respectively, and their percentual proportions increased with the increase of the total pigment content. The per cent of the yellow components decreased

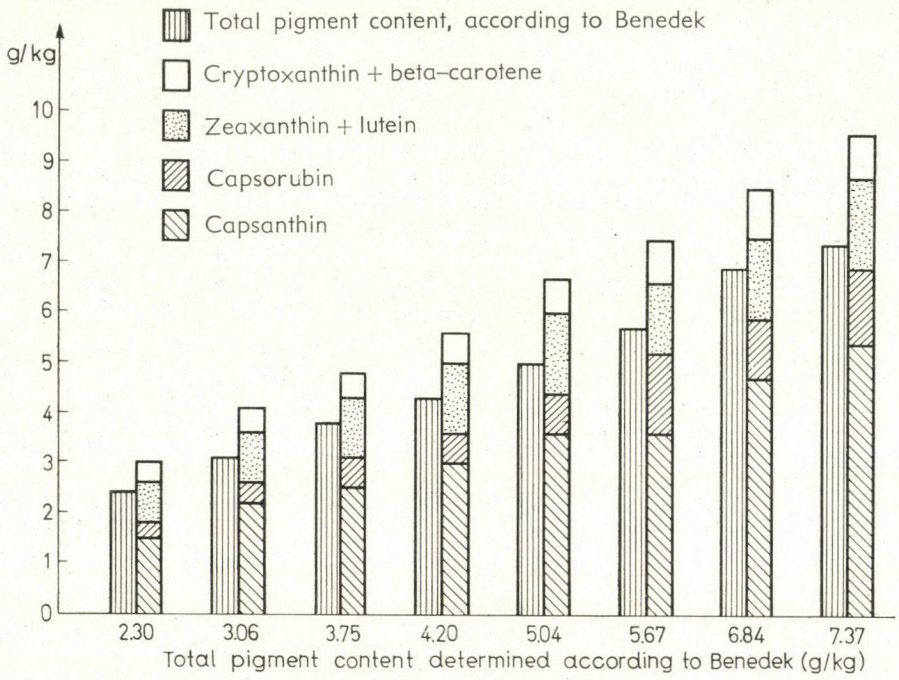

Fig. 12. The pigment contents of paprika as determined by Benedek's procedure and the thin layer chromatography method

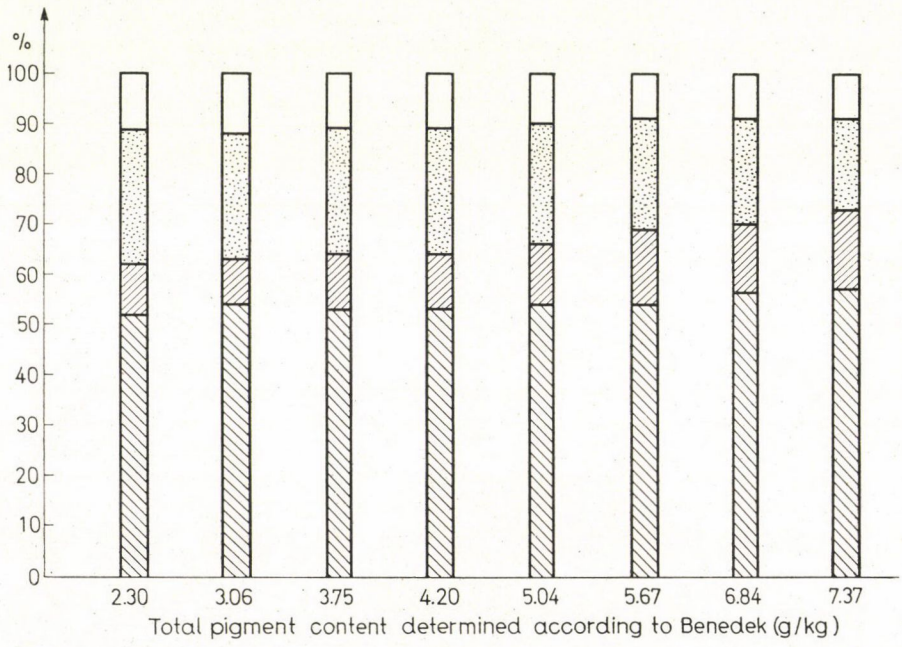

Fig. 13. Percentual distribution of the paprika pigment components isolated by thin layer chromatography

with increasing total pigment content; while the total of the red components amounted to 60—73%, that of the yellow components to 37—25% in the rising sequence of the total pigment content.

Table 4

Comparison of pigment determined in powdered paprika samples by thin layer chromatography and by Benedek's method

The mean values ($\bar{x}$), standard deviation (s) and the coefficient of variation (v) were calculated each from five replicates

	Total pigment content according to Benedek (g/kg)	Measurements by thin layer chromatography						Beta-carotene + cryptoxanthin (6)
		Total red components (1)	Total yellow components (2)	Total red + total yellow, calculated as (1) + (2)	Capsanthin (3)	Capsorubin (4)	Zeaxanthin + lutein (5)	
$\bar{x}$	2.30	1.77	1.09	2.87	1.49	0.29	0.74	0.34
s	0.067	0.058	0.038	0.155	0.032	0.001	0.016	0.005
v	2.92	3.32	2.83	5.41	2.18	3.52	2.26	1.69
$\bar{x}$	3.06	2.56	1.52	4.10	2.17	0.38	1.02	0.49
s	0.095	0.083	0.045	0.113	0.080	0.001	0.038	0.012
v	3.12	3.28	2.98	2.76	3.73	4.23	3.77	2.52
$\bar{x}$	3.75	2.99	1.72	4.72	2.49	0.51	1.20	0.52
s	0.151	1.104	0.049	0.198	0.061	0.002	0.085	0.015
v	4.03	3.49	2.89	4.21	2.47	3.18	7.14	3.03
$\bar{x}$	5.67	5.24	2.35	7.60	4.18	1.06	1.63	0.72
s	0.225	0.146	0.105	0.387	0.211	0.035	0.050	0.030
v	3.97	2.77	4.49	5.10	5.05	3.36	3.11	4.17
$\bar{x}$	7.37	6.94	2.57	9.52	5.59	1.54	1.83	0.83
s	0.325	0.258	0.127	0.386	0.179	0.068	0.083	0.027
v	4.42	3.72	4.97	4.06	3.22	4.46	4.58	3.36

The above relations suggest that there is an equilibrium between the red and yellow pigment components.

Further experiments with the thin layer chromatography method are scheduled to clarify the changes of colour components due to the storage of the raw material, and processing technology or storage of the finished product.

*

The authors are indebted to Associate Professor Dr. J. Szabolcs and his colleagues (Department of Chemistry, Medical University, Pécs) for supplying the isolated standard paprika pigment components and to the firm Hoffmann-LaRoche (Basel) for supplying the carotenoid preparations.

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A METHOD FOR THE DETERMINATION OF DEHYDROGENASE ACTIVITY OF METHANE ASSIMILATING BACTERIA

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Facultative methane assimilating bacteria grown on methane as a sole carbon and energy source were examined for a possible relationship between their dehydrogenase activity and growth rate. Dehydrogenase activity was measured by the discoloration of methylene blue added to the system as an indicator.

First the conditions of culturing (temperature and pH of the growth medium, dissolved oxygen concentration, bacterial count of the inoculum) and the applied concentrations of methylene blue were tested for their influence on the time of reduction of the indicator by the enzyme. This was not altered by the initial temperature of 20 and 37 °C of the medium incubated, neither by the concentration of methylene blue in a range of 100—400 mg/100 ml, but changed considerably with the level of dissolved oxygen in the medium. The latter relationship was, however, non-linear, as under the given conditions the rise of the oxygen concentration could only take place at the cost of reducing the methane level in the system.

The time of discoloration varied with the pH of the medium and in the range tested (6.5—7.4), pH 7.1 was the least favourable for the reaction. The concentration of bacteria in the system also influenced the time of discoloration considerably.

On the basis of the above observations, the conditions of dehydrogenase assay were established as follows: pH 7.2 in the medium; CH_4 and air mixture, 50 : 50; bacterial concentration of the inoculum 3×10^{-2} g/l; methylene blue concentration 200 μg/100 ml.

The correlation between the enzyme activity, measured by its reducing effect, and the rate of bacterial growth was found to be highly significant. This suggests the suitability of the method for the assessment of the growth ability of bacterial inocula.

Reports on the microbiological use of gaseous hydrocarbons are still less numerous than those describing the propagation of bacteria in liquid hydrocarbons. Considering the novelty of the former topic, most experimental results have been conflicting and most conclusions are hypothetical.

Opinions are still divergent about the nature of the enzymes taking part in methane oxydation occurring in several stages and even the possible modes of methane metabolism are still inadequately known.

The first step of the process is particularly poorly understood. VAN der LINDEN and THIJSSE (1965), PERRY (1968), WHITTENBURY (1969), PROCTOR et al. (1969) and DAVIES and WHITTENBURY (1970) believe that it occurs with the participation of an oxygenase enzyme, not specific as regards the chain length of the hydrocarbon. Cell yield on methane is about twice as high as on equivalent methanol, suggesting that the reaction step $CH_4 \longrightarrow CH_3OH$

is an energy producing process. Two electrons are released on the formation of methanol. The values of oxygen consumption observed with labelled O_2 have also indicated the presence of oxygenase.

FUHS (1961) and CLABHUHA (1947), in contrast, suppose peroxydase activity in the first stage of methane oxidation.

According to HARRINGTON and KALLIO (1960), VAN der LINDEN and THIJSSE (1965), the transformation of methanol to formaldehyde is catalysed by peroxidase. However, KANEDA and ROXBURGH (1959), JOHNSON and QUAYLE (1965) and WHITTENBURY (1969) have demonstrated the presence of alcohol dehydrogenase activity; while HARRINGTON and KALLIO (1960) found alcohol peroxydase activity in the oxidation of methanol to formaldehyde by Pseudomonas methanica. The latter two authors ascribed the controversial results to the possibility of methanol oxidation in two different ways, either by DPN-bound alcohol dehydrogenase or catalase-bound peroxydase.

VAN der LINDEN and THIJSSE (1965) and HARRINGTON and KOLLIO (1960) demonstrated formaldehyde dehydrogenase during the oxidation of formaldehyde and believed it to be substrate specific. JOHNSON and QUAYLE (1964), in contrast, observed the activity of a broad range aldehyde-dehydrogenase.

Alcohol and aldehyde dehydrogenases were isolated from bacteria grown in longer chain hydrocarbons (C_6, C_7, C_8, C_{10}) (JACOBY, 1957; BAPTIST et al., 1962; AZOULAY & HEYDEMAN, 1963; LEAVITT, 1967). Both these dehydrogenases have a broad range of action. Leavitt states in his patent description that the cell enzyme, not separated into alcohol and aldehyde dehydrogenases, is able to catalyze the oxidation of not only the alcohols and aldehydes of the same chain length as the hydrocarbon serving as carbon source, but those of different chain length as well.

In the case of non-obligatory methane oxidizing organisms which grow on many members of the paraffin series, it can always be supposed that enzymes of broad substrate specificity participate both in the oxidation of the hydrocarbon to the appropriate alcohol and in the oxidation of the alcohol to aldehyde.

The strains used in this study have been facultative methane assimilating bacteria, growing in addition to methane also on hydrocarbons of greater chain length and on conventional growth media as well. It was, therefore, supposed that the dehydrogenases played an active role in the initial steps of methane assimilation by these organisms.

To elaborate a dehydrogenase activity assay which would suit the given conditions, the concentrations of methylene blue, the temperature and pH of the growth medium, the bacterial contents of the sample and the dissolved oxygen level each were tested for effect on the discoloration time of the redox-indicator.

1. Materials and methods

1.1. Bacterial cultures

A strain screened from the microbial flora of the soil of natural gas areas as well as from crude natural gas was used throughout. Adaptation of the strain for morphological and methane assimilation studies was carried out according to HALÁSZ and SIMEK (1968).

1.2. Growth medium

The strain was cultured in a sterile mineral salt medium in a methane + air atmosphere. $(NH_4)_2SO_4$ was added as a nitrogen source, being the most advantageous for the strain. No trace elements were added to the growth medium, because in preliminary experiments (HALÁSZ, 1969) they have been shown to be indifferent for the growth rate and cell yield of the bacterial strain. The composition of the medium is shown in Table 1.

Table 1

Composition of the synthetic liquid growth medium

$MgSO_4$	0.10 g/l
Na_2HPO_4	0.60 g/l
KH_2PO_4	0.14 g/l
NaCl	1.00 g/l
$(NH_4)_2SO_4$	0.60 g/l

1.3. Methane + air atmosphere

Gas mixtures of the required composition were prepared with an apparatus (Figure 1) described previously by HALÁSZ (1969). The appropriate methane + air atmosphere of the culturing flasks was provided by passing through the gas mixture for 15 minutes.

1.4. Cultivation of bacteria

The bacteria were grown in shake cultures, using 500 ml flasks closed by rubber stoppers. Methane + air were introduced into the system by means of injection needles (one No. 200×150 and one No. 2). After the gas flow was stopped the needles were extracted, leaving the elasticity of the rubber material to provide for an airthight closing.

250 ml growth medium was placed in each flask and the gas mixture was passed through at a rate of 150 litre gas per hour, for 15 minutes.

For sampling, the flasks were turned upside down and the sample was withdrawn by means of a syringe, using a No. 2 needle.

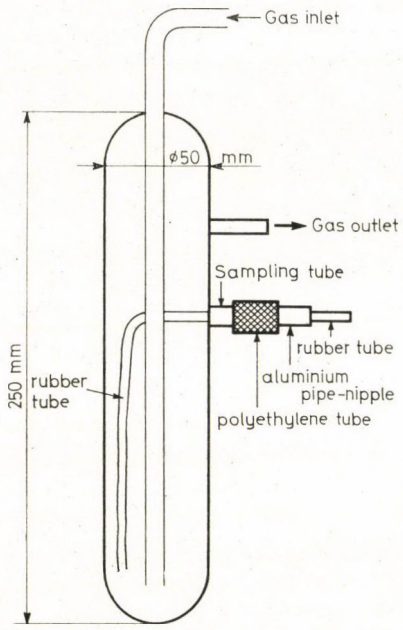

Fig. 1. Glass fermentor with sampling tube

1.5. Assessment of bacterial multiplication

Bacterial growth was followed up by turbidimetry (HALÁSZ and SIMEK, 1968), using an Orion-Gyem extinctiometer.

1.6. Determination of dehydrogenase activity

It was our aim to determine the dehydrogenase activity from the colour change of a redox-indicator. Of the indicators tested (methylene blue, methyl violet, phenol red, safranine, triphenyltetrazolium chloride, resazurin) only methylene blue and resazurin changed their colour under the given conditions, but the preliminary observation that the colour change of the latter was unrelated to the rate of bacterial growth left methylene blue as the only indicator suited for the purpose.

The reduction of methylene blue was followed up by photometry, using an Orion-Gyem extinctiometer. 0.2 ml of an aqueous solution of methylene

blue (25 mg/100 ml) was added to the bacterial suspension under methane + air atmosphere and the test tube was closed with a rubber stopper. The sample was incubated at 37 °C and tested every 10 minutes for extinction at 640 nm which corresponds to the absorption maximum of methylene blue.

2. Results

2.1. Effect of the concentration of methylene blue on the time of discoloration

Care was taken to choose as the initial concentration of methylene blue one with measurable extinction. The effect of methylene blue was, therefore, tested in a concentration range of 100—400 μg/100 ml.

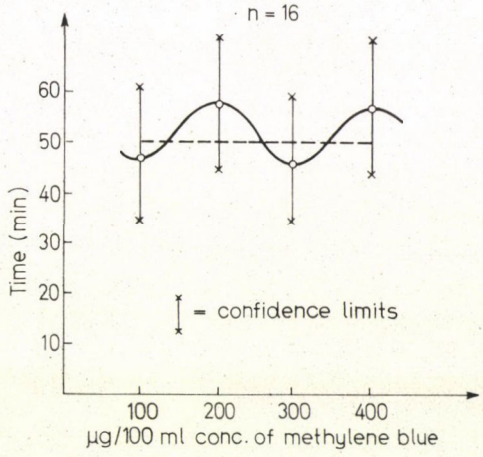

Fig. 2. Discoloration time of methylene blue as a function of indicator concentration

Table 2

Concentration-dependence of the discoloration time of methylene blue

Time of discoloration (minutes)

Concentration of methylene blue μg/100 ml			
100	200	300	400
47	56.6	45.5	55.5

Analysis of variance

	Degree of freedom	Sum of squares	Variance	F
Intergroup variance	3	11.43	3.8	
Intragroup variance	32	128.57	4.02	1.42

The intergroup differences are not significant.

An analysis of variance of the experimental data showed no significant difference between the discoloration times measured at 100, 150, 200, 300 or 100, 200, 300, 400 μg/100 ml concentrations of methylene blue (Table 2).

2.2. Effect of temperature of the medium on discoloration time

Discoloration times were compared in samples of room temperature and 37 °C, respectively, inoculated with identical cell counts and the difference found was not significant (Table 3).

Table 3

Temperature-dependence of the discoloration time of methylene blue

Time of discoloration (minutes)

Temperature of sample	
20 °C	37 °C
41	45

$s_m t = 5.78$ min.
($t = 0.89446$; the two average values do not differ significantly)

2.3. Effect of the pH of the medium on discoloration time

Discoloration time was measured at the optimum pH and at pH levels to which the shaken cultures were reduced during the interval between two samplings, at 5 and 24 hours after inoculation, respectively. Thus the pH levels tested were 6.5, 7.0, 7.1, 7.2, 7.3, 7.4. The results are shown in *Fig. 3*.

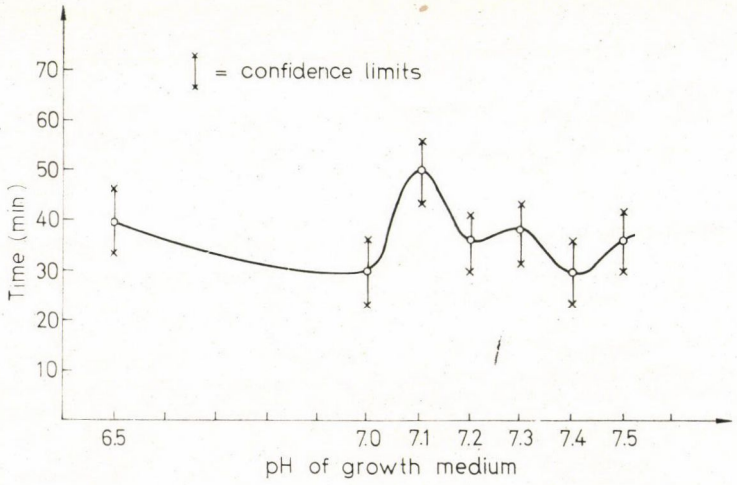

Fig. 3. Discoloration time of methylene blue as a function of the pH

Table 4

pH-dependence of the time of discoloration of methylene blue

Time of discoloration (minutes)

7.0	7.4	7.2	7.5	7.3	6.5	7.1
30	30	36	36	38	40	30

Analysis of variance

	Degree of freedom	Sum of squares	Variance	F
Intergroup variance	6	17.91	2.99	
Intragroup variance	35	24.11	0.69	4.43

Intergroup differences are significant ($P \geq 99\%$)

pH	6.5	7.0	7.1	7.2	7.3	7.4
7.0	×					
7.1	×	×××				
7.2	×	∅	×××			
7.3	×	∅	×××	∅		
7.4	×	∅	×××	∅	∅	
7.5	×	∅	×××	∅	∅	∅

∅ = difference not significant
× = difference significant ($P \geq 95\%$)
×× = difference significant ($P \geq 99\%$)
××× = difference highly significant ($P \geq 99.9\%$)

The analysis of variance (Table 4) revealed that the discoloration times differed significantly at various pH levels.

pH 6.5 was significantly less favourable than pH 7.0 or 7.4 and pH 7.1 was significantly less favourable than any of the pH levels tested. The difference between the rest of the pH values tested was not significant, as established by t test.

2.4. Effect of the concentration of dissolved oxygen in the growth medium on discoloration time

The effect of various concentrations of dissolved oxygen was studied by using substrates inoculated with identical cell concentrations and to saturate the medium, gas mixtures of different composition were bubbled through it and the discoloration time was measured.

Discoloration times as a function of the % oxygen content of the gas mixture are shown in Fig. 4.

Mathematical-statistical evaluation of the experimental data revealed highly significant differences between the discoloration times measured at various oxygen concentrations (Table 5).

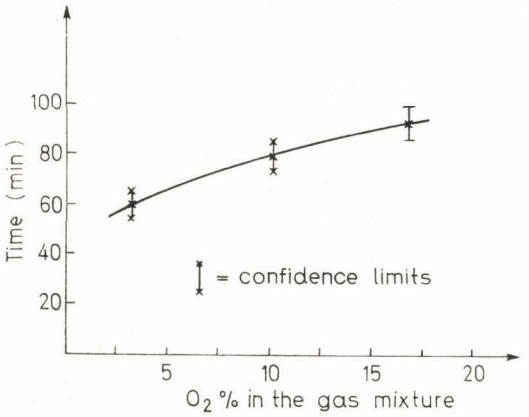

Fig. 4. Discoloration time of methylene blue as a function of the O_2 concentration in the methane + air gas mixture

Table 5

Dependence of the discoloration time of methylene blue on the O_2 *concentration of the gas mixture*

Time of discoloration (minutes)

Gas mixture O_2 level (%)		
3.33	10	16.6
60.9	81.6	93.3

Analysis of variance

	Degree of freedom	Sum of squares	Variance	F
Intergrup variance	2	3550.5	1775.25	
Intragroup variance	15	2313	153.5	11.51

Intergroup differences are significant ($P \geq 99\%$)

Significance of difference between methylene blue discoloration times measured at various levels of O_2 in the gas mixture.

	3.33	10
10	×	
16.6	××	∅

n = 6

∅ difference not significant
× difference significant ($P \geq 95\%$)
×× difference significant ($P \geq 99\%$)

Samples saturated with a gas mixture containing 3.3 per cent by volume of oxygen discoloured in a significantly shorter time than those saturated with a mixture of 10 or 16.6% oxygen. No significant difference was found between the discoloration times measured at 10 and 16.6% oxygen contents.

2.5. *Effect of the bacterial concentration on the discoloration time of methylene blue*

This was tested in a concentration range of $1.5-7\times10^{-2}$ g bacteria per litre. Discoloration times were measured in samples inoculated with different cell counts from the same culture and saturated with a gas mixture of uniform composition. The results are shown in Fig. 5.

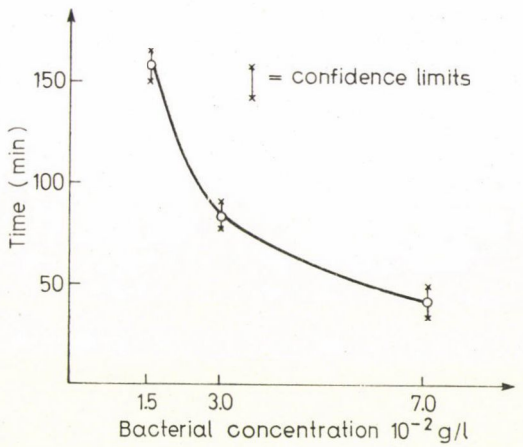

Fig. 5. Discoloration time of methylene blue as a function of bacterial concentration in the culture

The analysis of variance showed that the discoloration times varied significantly in the bacterial concentration range tested (Table 6).

The difference was highly significant between the values measured at bacterial concentrations of 1.5×10^{-2}, 3×10^{-2} and 5×10^{-2}.

2.6. *Relationship between the dehydrogenase activity assessed from the reduction of methylene blue and the growth rate*

The dehydrogenase activity was measured under standard conditions, keeping the bacterial concentration of the suspension at 3×10^{-2} g/l, its pH at 7.2 and its dissolved oxygen content at the level corresponding to 10% oxygen in the gas atmosphere. Though in the concentration range tested, methylene blue had no influence on discoloration time, it was applied uniformly at a level of 200 μg/100 ml.

Cultivating the bacteria in a closed fermentor system, the time of reduction of methylene blue was measured in samples of the freshly inoculated medium and simultaneously the multiplication of the organism in the growth

Table 6

Dependence of the time of discoloration of methylene blue on the bacterial concentration of the sample

Time of discoloration (minutes)

Bacterial concentration of the sample 10^{-2} g/l		
1.5	3	7
158	81.5	46.6

Analysis of variance

	Degree of freedom	Sum of squares	Variance	F
Intergroup variance	2	87,087.6	43,543.8	
Intragroup variance	36	8,975	249.3	17.47

Intergroup differences are significant ($P \geq 99\%$)

Significance of differences between the methylene blue discoloration times measured at various bacterial concentrations. Bacterial concentration 10^{-2} g/l

	1,5	3
3	×××	
7	×××	×××

n = 10
××× = difference highly significant ($P \geq 99.9\%$)

medium was followed up. Expressing the time of the reduction of methylene blue in minutes and the bacterial growth as per cent concentration increase from 0 to 60 minutes $= \frac{\Delta\%}{\text{time/min./}}$ the two values show the correlation presented in Fig. 6.

On converting the discoloration time of methylene blue into a rate value by dividing the hydrogen volume (3.76 μl H_2) equivalent to the amount of methylene blue in the sample by the time of discoloration in minutes, the curves shown in Fig. 7 were obtained.

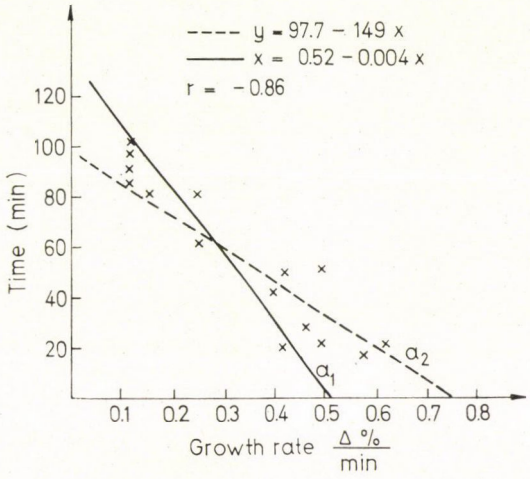

Fig. 6. Correlation between methylene blue discoloration time and bacterial growth rate. (pH = 7.2; bacterial conc. 3×10^{-2} g/l; O_2 level = 10%; methylene blue level = 200 μg/100 ml.)

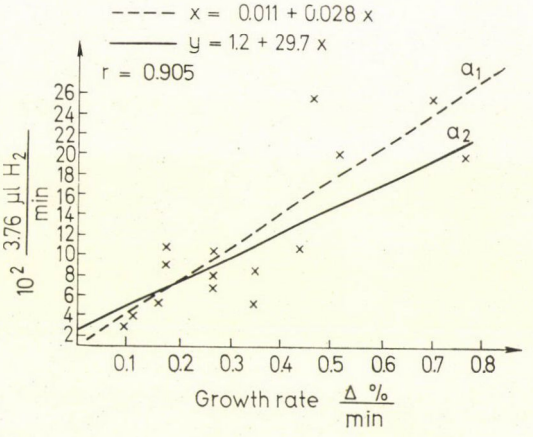

Fig. 7. Correlation between discoloration rate of methylene blue and growth rate of bacteria. (pH = 7.2; cell concentration 3×10^{-2} g/l; O_2 = 10%; methylene blue = 200 μg/100 ml.)

3. Conclusions

Davis and Yarbrough (1966) found that the time of discoloration depended on the concentration of methylene blue, but they used about 100 times higher concentrations of bacteria and only 4—16 times higher concentrations of methylene blue, thus the relative methylene blue concentration in this study was substantially higher. As can be seen from Table 3, the initial temper-

ature of the medium (20 and 37 °C) had no influence on the time of methylene blue discoloration. The pH of the growth medium, on the contrary, influenced it significantly, as demonstrated by analysis of variance.

Another factor affecting the discoloration time of methylene blue was the level of dissolved oxygen in the medium (Table 5), but the relationship between them was non-linear, because under the given conditions the elevation of the concentration of dissolved oxygen had to be effected at the cost of lowering the amount of methane, serving as a carbon and energy source in the system (Fig. 4).

As shown also by analysis of variance (Table 6), the discoloration times varied significantly with the bacterial concentration in the range of $1.5-5\times10^{-2}$ g/l.

Our method for dehydrogenase assay was elaborated on the basis of the above findings.

The pH of the growth medium was adjusted to 7.2; this is particularly important with re-gassed samples. The gas mixture was prepared from equal volumes of methane + air (50 : 50) and the bacterial concentration was established as 3×10^{-2} g/l. Although methylene blue has no influence on reduction in the concentration range of 100—400 μg/100 ml, its level was uniformly kept at 200 μg/100 ml. Bubble-free filling of the test tubes is imperative to obtain reliable data.

Discoloration was followed up by measuring the extinction values every 10 minutes during the first hour and subsequently every 30 minutes.

Examining by correlation analysis the relationship between the dehydrogenase activity measured by reduction of methylene blue and the growth rate, a highly significant correlation was found. The correlation coefficient of the discoloration time and growth rate was $r = -0.86$ and that between the discoloration rate and growth rate was $r = 0.905$, that is, they were significant at 95 and 99 % probability levels, respectively.

Since from the point of view of bacterial multiplication growth rates below $\frac{0{,}1\Delta\%}{\text{time}}$ are negligible, the corresponding methylene blue discoloration times viz. methylene blue reduction rates signified an unsatisfactory growth ability of the inoculum if the conditions of culturing were optimal.

*

The author is indebted to Miss Á. Baráth and Miss I. Pfeiffer for excellent technical assistance.

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RADURIZATION OF WHOLE EVISCERATED CHICKEN CARCASE

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The aim of the present experiments was to assess the means of extending the storage life of whole eviscerated chicken carcases by ionizing radiation at dose levels not impairing the sensory quality of the flesh.

Eviscerated whole carcases weighing 0.8—0.9 kg were sealed into Saran shrink foil and irradiated with 0.2—0.5 Mrad gamma rays from a ^{60}Co radiation source. Foilpacked carcases exposed and not exposed to irradiation were then stored at 0 . . . . . + 4 °C. Lots of 25 carcases were treated at each dose level. During storage, the deterioration of the sensory quality as well as the change of the total viable cell count were followed up. At given intervals of the storage period, unspoiled raw samples as well as cooked and fried samples were subjected to sensory evaluation by a jury of 8 judges.

Irradiation with 0.2—0.5 Mrad was found to extend the keeping quality of chicken carcases in cold storage 2—3 times, viz. at least for 10—15 days without notable deterioration of the sensory quality. Preliminary examinations performed in the laboratories of the National Poultry Industry Company showed the above radiation treatment to practically eliminate the salmonella infection of the carcases.

These findings are in good agreement with the literary data and with the results of our own preliminary investigations as well.

Economic calculations in this country and literary data from abroad unequivocally suggest a costs estimate of about Ft 0.4 for 1 kg product with a ^{60}Co radiation source of 250 kCi activity used for radiation treatment.

Meat is one of the most important protein sources of the human diet Poultry flesh, superior in protein content to other meats, is a food item of outstanding significance. SCOTT (1956) found the protein content of boiled chicken flesh 25—30%, with the edible viscera disregarded. The protein content naturally depends on the specific part of the carcase and on the method of processing as well. The protein contents of beef, pork and mutton are 21—27, 23—24 and 21—24%, respectively. The advantages of poultry flesh over meats are its low caloric value, high essential lipid and amino acid contents and easy digestibility.

The recent abrupt expansion of broiler chicken production has been of great importance not only from the economical, but also from the nutritional point of view. Increase of the production and export volumes has stressed the necessity of extending the storage life of eviscerated whole chicken carcases.

Though quick freezing of the poultry carcase has resolved the problem of long-term preservation, consumers generally prefer the non-frozen product. But simple cold storage in itself does not provide for a reasonable storage life.

This has initiated investigations into the combination of radiation preservation and cold storage for the purpose of extending the keeping quality of commercial ready-to-cook chickens.

The radiation dose required to stabilize the microbiological condition of the chicken carcase at room temperature is relatively high and likely to affect the sensory qualities of the product. Cold storage, however, enables a reasonable reduction of the radiation dose and the combination of the two methods is more economic and less energy-requiring than deep freezing.

The combination of radurization with cold storage results in a considerable extension of the storage life (INGRAM, 1959). At low temperatures the metabolic processes slow down and enzyme activity becomes suppressed.

The microflora of meat consists primarily of bacteria, these microorganisms being responsible for its spoilage. The bacterial flora of meat is very variable and psychrophilic organisms play an important role (PETERSON & GUNDERSON, 1960).

Certain components of the microflora of meat are highly sensitive to irradiation, while other components, particularly the micrococci, are radiation resistant. The minimum effective dose of irradiation depends on the actual radiation tolerance of the microorganisms, whereas the maximum dose is limited by changes in sensory quality and costs. Various radiation doses were, therefore, examined for their influence on storage life and sensory qualities of the whole eviscerated chicken carcase.

1. Materials and methods

1.1. Preparation of the test material

Carcases of freshly slaughtered and gutted chickens, weighing 800—900 g, obtained from the Budafok Poultry Processing Plant of the National Poultry Industry Company were used. The carcases were packed one by one in Saran shrink foil; the foil pouches were then evacuated, sealed and allowed to shrink. Irradiation was carried out immediately after the completion of the packing procedure.

1.2. Irradiation

A ^{60}Co gamma radiation source of 50,000 Ci nominal activity, available at this institute, was used for radiation treatment. At the place of exposure, temperature was 12—15°C and relative humidity was 60%. The experiment was performed in two series. In the first series, the samples were irradiated with 0.26 ± 0.02 and 0.51 ± 0.07 Mrad, at a dose rate of 0.36 Mrad/hour.

The dose levels applied in the second series were 0.22 ± 0.04 and 0.39 ± 0.06 Mrad, at a dose rate of 0.20—0.22 Mrad/hour. The dose rate at the place of exposure was determined by means of Fricke's iron(II)sulfate solution (WEISS et al., 1955).

1.3. Storage experiments

The samples were stored at 0 . . . . . + 4 °C temperature. Since at that temperature the non-irradiated control samples deteriorated relatively rapidly, part of them were stored at —28 °C to be available for comparison in the later phase of the experiment. At each dose level, 20—25 samples each were used for the experimental and control lot.

The condition of the samples was checked regularly during the period of storage. Deterioration was judged by unpleasant smell and such samples were removed from the lot. To facilitate judgement of the smell, the pouch was punched with a needle. Recontamination of the carcase through the punched hole was never noted.

The relative storage life of the samples was assessed by means of spoilage curves plotted from the spoilage data. The period during which 5, 10 or 20% of the control samples deteriorated was arbitrarily considered as unit time. The time required for the irradiated samples to deteriorate in similar proportion was related to the control values.

1.4. Determination of total viable cell count

Muscle specimens secured for bacteriological examination were weighed with an accuracy of ± 10 mg under sterile conditions. The samples weighed about 20 g, with approx. 900 square mm surface. The weighed samples were homogenized each in 80 ml distilled water, using a M.S.E. homogenizer at 14,000 rpm for 3 minutes. For each dose level two samples, one from the thigh, one from a dorsal muscle, were tested. The above homogenate served as stock suspension for dilutions and inocula. Inoculations were made in 3 replicates for each dilution level. For pour-plate examination universal medium*, with 2% agar-agar added for solidification, was used. The inoculated media were incubated at 28—30 °C. Colonies were counted after incubation for 5 days.

1.5. Sensory evaluation

Sensory evaluation was made at regular intervals during the period of storage. Simultaneously, raw samples were examined for eventual off-odour due to radiation treatment. Using the habitual kitchen technology, one

* Composition: Sweet whey 200 ml, yeast extract (1 : 10) 100 ml, meat extract 4 g, pepton 2 g, water 700 ml.

sample was boiled with vegetables added, to obtain chicken broth and cooked meat, the other sample was fried. The broth was prepared in a self-pressure cooker (trade mark "Kukta") by boiling for 20 minutes. Frying was done in pork fat in an electric oven for 45 minutes at 230 °C.

Sensory evaluation was carried out by a jury of 8 judges. Depending on the type of processing of the sample, colour, flavour and odour were evaluated by ranking in the following seven-score system:

7 scores: excellent
6 scores: good
5 scores: medium quality
4 scores: acceptable
3 scores: objectionable
2 scores: highly objectionable
1 score: spoiled

Evaluation of the scores was made by ranking according to KRAMER (1960).

2. Results

2.1. Storage life

The storage life of the gutted chicken carcase at refrigerator temperature (0 . . . . . + 4 °C) is 4 to 7 days. The first sign of deterioration is an unpleasant odour resulting primarily from the activities of microorganisms and tissue enzymes. Subsequently visible colonies of bacteria appear on the carcase with simultaneous glossy or perhaps mucinous appearance of the surface and copious exudation of juice. The proteolytic activities of microorganisms (Clostridium, Bacillus, Pseudomonas organisms and certain species from other genera) and tissue enzymes may result in complete decomposition of the carcase. Breakdown of the protein molecule into peptides, amino acids or other decomposition products (mercaptanes, NH_3, etc.) informs to a certain extent on the type of spoilage (SALISBURY & CRAMPTON, 1960). Eventual unusual odour due to radiation treatment does not interfere with the judgement of spoilage.

The tiny hole made by punching the foil with a needle proved sufficient to assess the odour. Samples found deteriorating were removed immediately. Non-irradiated samples deteriorated relatively rapidly as compared to the irradiated ones, within 6—7 days spoilage reached already 100%. Taking a 5% level of spoilage into account which took 5.2 days in the control series, irradiation with 0.26 $\pm$ 0.02 Mrad prolonged the storage life of the product to 8.4 days, while 0.51 $\pm$ 0.07 Mrad to 11.6 days. In the other experimental series, treatment with 0.22 $\pm$ 0.04 Mrad extended the storage life of the samples to 5 days as contrasted to 2.4 days in the untreated control lot (Figs 1, 2).

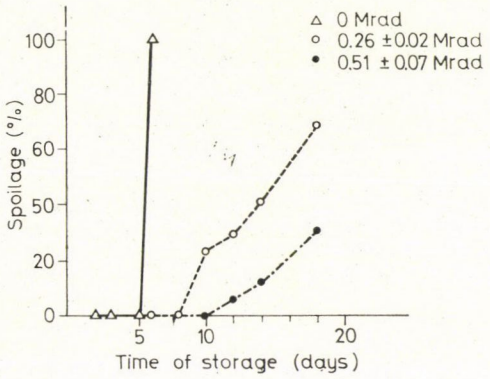

Fig. 1. Spoilage of foil-packed eviscerated chicken carcase as a function of radiation dose and time of storage at 0 . . . . . + 4 °C (1st experimental series)

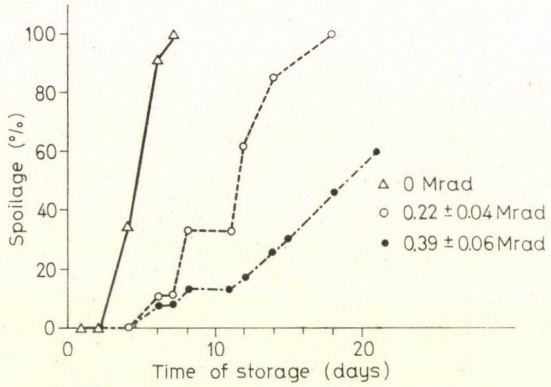

Fig. 2. Spoilage of foil-packed eviscerated chicken carcase as a function of radiation dose and time of storage at 0 . . . . . + 4 °C (2nd experimental series)

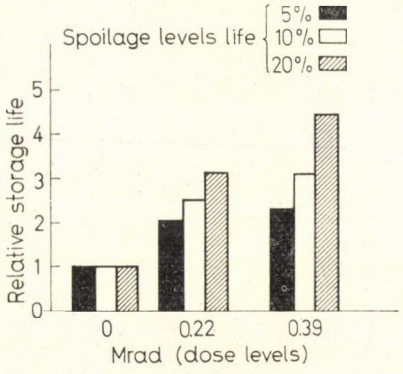

Fig. 3. Relative storage life of foil-packed eviscerated chicken carcase at 0 . . . . . + 4 °C as a function of the irradiation dose as assessed at spoilage levels of 5, 10 and 20%, respectively (1st experimental series)

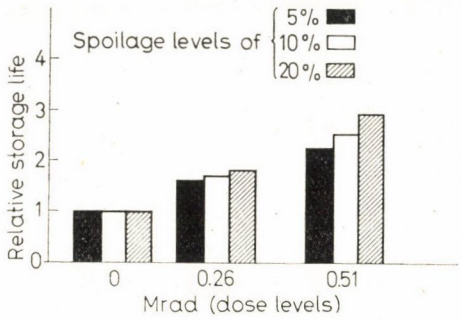

Fig. 4. Relative storage life of foil-packed eviscerated chicken carcase at 0 . . . . . + 4 °C, as a function of the irradiation dose, assessed at spoilage levels of 5, 10 and 20%, respectively (2nd experimental series)

Relative keeping qualities (see paragraph 1.3) were assessed from the above data. At 5% spoilage level, irradiation with 0.2—0.5 Mrad extended the relative keeping quality 2—3 times, whereas at 20% spoilage level 3—5 times (Figs 3, 4).

2.2. Changes of viable cell count

Initial viable cell count was found to be 10^5—10^6/g. The various components of the meat microflora differ in sensitivity to ionizing radiation. The microorganisms most resistant to radiation are the yeasts, but as they constitute a very low proportion, 1—2% (KISS & FARKAS, 1965) of the meat microflora, their activity may be considered immaterial. Pseudomonas organisms constitute a substantial proportion of the microflora, but they are very sensitive to irradiation (THORNLEY, 1957). Mention should be made also of the psychrophilic microorganisms, the radiation sensitivity of which varies with the temperature of irradiation. To prevent eventual changes in sensory quality radiation treatment at low temperatures seems promising. High radiation tolerance at low temperature has been nevertheless, also described (MECURI et al., 1967) as a factor likely to reduce the effectiveness of radiation treatment.

Results of the first series of experiments suggested that irradiation with 0.25 and 0.51 Mrad immediately reduced the viable cell count by 1—2 and 2.5—3 orders, respectively. In the untreated samples the viable cell count reached a level of 10^9/g already on the 9th day. The viable cell count of the irradiated samples rose at a slower rate, being still at a level of 10^4—10^7/g on the 8th—10th day. Irradiation at a dose level of 0.51 Mrad was sufficient to keep the viable cell count at a level of 10^6—10^7/g for almost 3 weeks (Fig. 5).

In the second experimental series, the first determination of viable cell count was made 24 hours after exposure. Irradiation with 0.22 or 0.39 Mrad reduced the viable cell count by hardly one order of magnitude as compared

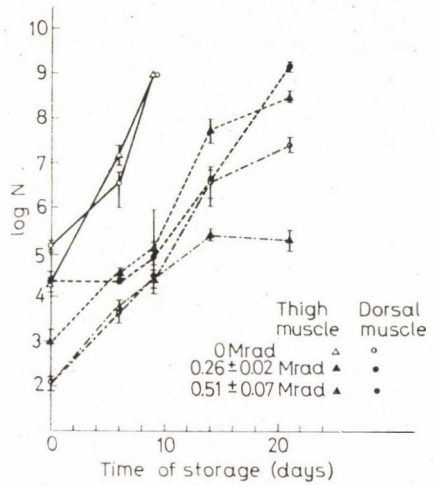

Fig. 5. Viable cell counts of the foil-packed eviscerated chicken carcase as a function of the dose of irradiation and time of storage at 0 . . . . . + 4 °C. The vertical lines show standard deviations about the mean (1st experimental series)

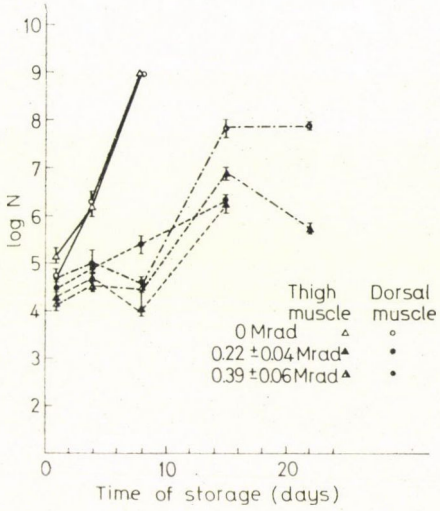

Fig. 6. Viable cell counts of the foil-packed eviscerated chicken carcase as a function of the dose of irradiation and time of storage at 0 . . . . . + 4 °C. The vertical lines show standard deviations about the mean (2nd experimental series)

to the non-exposed controls. But the cell counts of the radiation-treated samples remained practically unchanged for 8 days and did not rise above 10^7—10^8/g for further 14 days (Fig. 6).

No unambiguous correlation was found between the increase of cell count and the development of unpleasant odour. But when the samples started

to smell (20% spoilage), the total viable cell counts were found to vary in the range between 10^5-10^7/g in the experimental and control lots alike. This suggested that radiation treatment caused no notable alteration in the character of the decomposition of the eviscerated chicken carcase.

Relatively low radiation doses have been shown to effect elimination also of salmonellae (LICCIARDELLO et al., 1968; MOSSEL et al., 1968; IDZIAK & INCZE, 1968). Informative experiments were carried out in the Research Department of the National Poultry Industry Company to assess to what extent the applied doses of irradiation would affect salmonellae. The results have been promising, as exposure at a 0.39 Mrad dose level reduced the total count of salmonellae by 5—6 orders.

2.3. Sensory evaluation

During the storage of meat, various biochemical reactions are taking place which can be controlled by appropriate alteration of the conditions of storage. Also radiation treatment may give rise to various compounds the

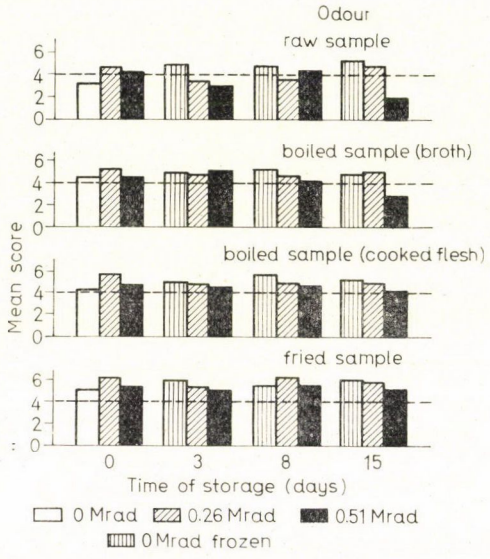

Fig. 7. Sensory evaluation of foil-packed eviscerated poultry carcase. Average scores for the *odour* of raw and processed samples (broth, cooked and fried flesh) as a function of the dose of radiation treatment and time of storage at 0 . . . . . + 4 °C. From the 3rd day of storage on, non-irradiated frozen samples stored at —28 °C served as control. The dashed line signifies the level up to which the quality of the samples was acceptable (4 scores) (1st experimental series)

quantities of which vary with the type and quality of the meat and with the conditions of storage and radiation treatment as well (COLEBY, 1959). The liability of various meats to alterations of taste increases in the following

order: pork, chicken, mutton, veal, beef (URBAIN, 1965). Very small quantities of volatiles due to radiation treatment such as hydrogen sulfide, mercaptane, indole, isovaleraldehyde, etc. may impart an unpleasant odour to the meat (LÜCK & KOHN, 1960; PALMIN, 1961). Such changes of odour and taste often vanish during cooking or frying (PALMIN et al., 1959).

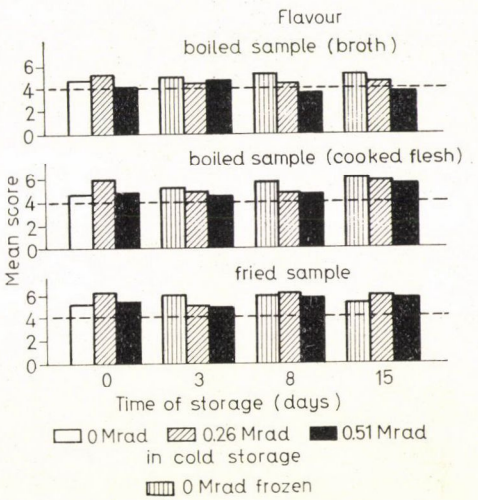

Fig. 8. Sensory evaluation of foil-packed eviscerated chicken carcase. Average scores for *flavour* of boiled (broth, cooked flesh) and fried samples as a function of the dose of radiation treatment and the time of storage at 0 . . . . . + 4 °C. After the 3rd day of storage, non-irradiated frozen samples, stored at —28 °C served as controls. The dashed line signifies the level up to which the quality of the samples was acceptable (4 scores) (1st experimental series)

Consistently with the literary data (COLEBY et al., 1960; RHODES, 1965) the radiation-treated chicken carcases showed a pale pink discoloration as compared to the untreated controls. But this change did not affect the quality of the carcase in any way, being imperceptible to the eye except by direct comparison with non-irradiated carcases. A similar colour change was noted also in previous experiments on pork cuts (KISS et al., 1969).

As the colour difference of treated and untreated raw samples was supposed to prejudice the judges in evaluating odour, we modified the organoleptic evaluation scheme proposed by MACLEOD and his co-workers (1969) in that smelling had to be performed in a dark room illuminated by weak red light only to exclude prejudice by visual impression. The jury consisted of 8 judges. Taste and odour were scored separately for the broth and boiled meat. Average scores are shown in Figs 7—10 and rank sums calculated from the scores in Figs 11 and 12.

It should be noted that owing to the rapid spoilage of the non-irradiated control samples, they were used for comparison only initially, being later replaced by quick frozen samples of unirradiated carcases.

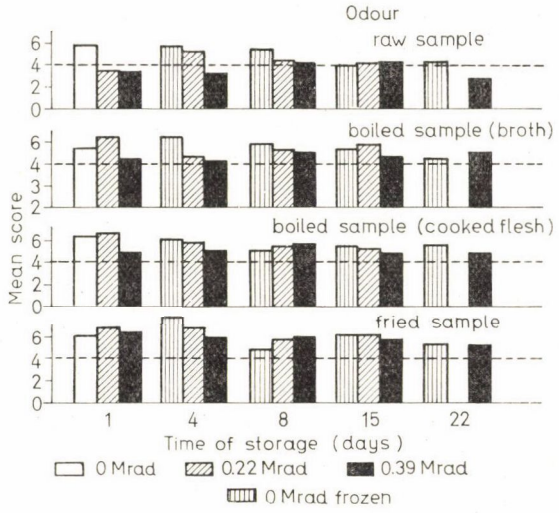

Fig. 9. Sensory evaluation of foil-packed eviscerated poultry carcase. Average score for the *odour* of raw and processed samples (broth, cooked and fried flesh) as a function of the dose of radiation treatment and the time of storage at 0 . . . . . + 4 °C. After the 3rd day of storage, non-irradiated frozen samples stored at —28 °C served as control. The dashed line signifies the level up to which the quality of the samples was acceptable (4 scores) (2nd experimental series)

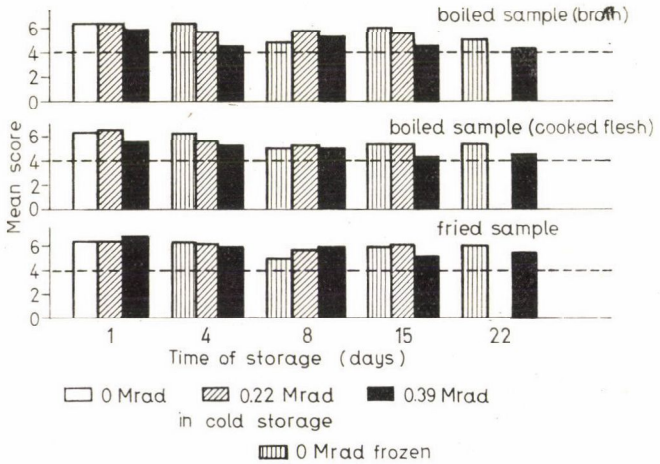

Fig. 10. Sensory evaluation of foil-packed eviscerated chicken carcase. Average scores for *flavour* of boiled (broth, cooked flesh) and fried samples as a function of the dose of radiation treatment and the time of storage at 0 . . . . . + 4 °C. After the 3rd day of storage, non-irradiated frozen samples, stored at —28 °C served as controls. The dashed line signifies the level up to which the quality of the samples was acceptable (4 scores) (2nd experimental series)

As can be seen from the Figures, at the $\alpha = 0.01$ level there was no significant difference between the sensory qualities of the radiation-treated and untreated samples, though in most cases the average scores for samples irradiated with 0.5 Mrad were lower than those for samples untreated or treated with lower radiation doses. Difference between the samples was most pronounced in respect to the odour of raw meat and broth.

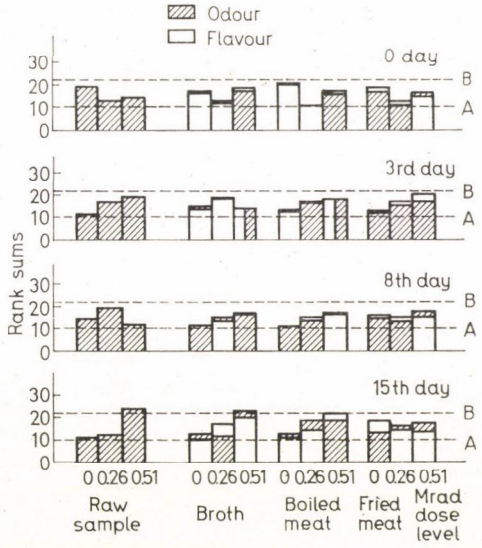

Fig. 11. Rank sums of scores for the sensory qualities of foil-packed eviscerated chicken carcase as a function of the dose of radiation treatment and time of storage. Evaluation of the scores of a jury of 8 judges was made by ranking according to KRAMER. The area enclosed by the dashed lines A and B represents the rank sums not differing significantly ($\alpha = 0.01$). After the 3rd day of storage, non-irradiated frozen samples stored at —28 °C were used as controls (1st experimental series)

3. Discussion

The experimental results clearly indicate that irradiation with 0.2—0.5 Mrad extended the storage life of refrigerated eviscerated chicken carcases 2—3fold, viz. to at least 10—15 days. This correlates well with the data of other authors (INGRAM & THORNLEY, 1959; MCGILL et al., 1959; THORNLEY et al., 1960; IDZIAK & INCZE, 1968) and with our previous findings (KISS & FARKAS, 1965) as well.

Comparison of the results of microbiological examinations of the carcases in two series of experiments permits the conclusion that the surviving microflora may undergo a certain regeneration on the first day following irradiation.

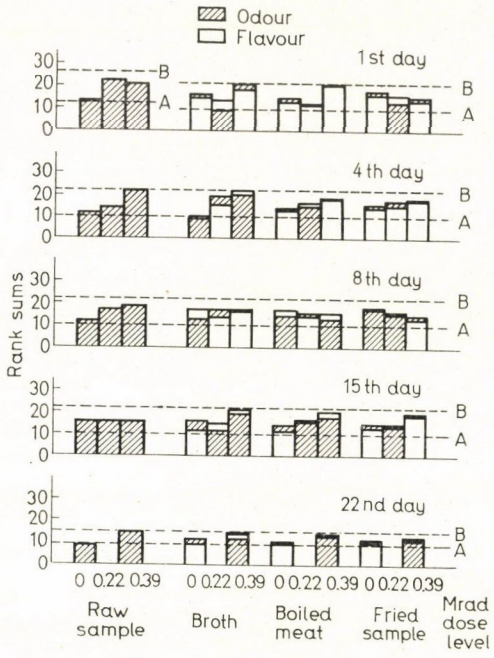

Fig. 12. Rank sums of scores for the sensory qualities of foil-packed eviscerated chicken carcase as a function of the dose of radiation and time of storage. Evaluation of the scores was made by ranking according to KRAMER. The jury of judges consisted on the first day of 10 persons, on further occasions of 8 persons. The area enclosed by the dashed lines A and B represents the rank sums not differing significantly ($\alpha = 0.01$). After the 4th day non-irradiated frozen samples, stored at —28 °C were used as controls (2nd experimental series)

The extended lag-phases in the curves obtained by plotting the viable cell counts of the radiation-treated samples versus storage time, as well as the slower rates of bacterial growth suggested the impairment of the surviving microflora.

Increasing, though not exponential reduction of the viable cell count with increasing irradiation doses as well as the differences noted in the two series of experiments may be interpreted as suggestive of the heterogeneity of the microflora of the chicken carcases in respect of radiation tolerance and a great variability of the microflora components from lot to lot (THORNLEY, 1966).

According to the results of sensory evaluation, the applied radiation doses had not notably affected the sensory qualities of the whole eviscerated chicken carcase. But the same findings suggest that radiation doses above 0.5 Mrad as well as cold storage beyond 15—20 days are likely to impair the organoleptic quality.

According to literary data (PHILLIPS et al., 1961; ZIMMERMAN & SYNDER, 1969), the slight colour change occurring on irradiation may be explicable by anaerobic transformation of part of the myoglobin to oxymyoglobin which has a more vivid red colour. In the present experiments, the presence of oxygen was excluded by packing the samples into the oxygen-impermeable Saran foil and by evacuation of the foil pouches.

The costs of radiation treatment have been assessed as Ft 0.4/kg for an 0.4 Mrad dose requirement, given a ^{60}Co radiation source of 250 kCi activity, operated in 3 shifts for an annual performance of 6000 tons (BALÁZS, 1968). This costs estimate is in good accordance with the results of similar calculations made in other countries, e.g. in the United Kingdom (RHODES, 1965) giving the costs of 0.15 Mrad dose requirement as d 1.4 and 0.6/kg at an annual output of 1900 and 7600 tons, respectively. RHODES (1965) stated these costs to be consistent with the criteria of economicalness under the prevailing conditions of the poultry industry in Great Britain.

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ASSIMILATION OF SORBIC ACID BY YEASTS

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Explanation was sought experimentally for the earlier demonstrated concentration decrease of, and cessation of inhibitory effect of, sorbic acid (SA) in growing yeast cultures. The concentration decrease of SA was found to take place also in resting cell suspensions incubated in phosphate buffer under aerobic conditions and certain yeasts, primarily *Procandida albicans*, *Pc. tropicalis* and *Candida claussenii*, were shown to assimilate SA as sole carbon and energy source for their growth. The growth rate depended in a complex manner on the concentration of SA, pH of the medium and presence of reduced glutathione (GSH) as well. With yeast species capable of metabolizing and assimilating SA, low intracellular concentrations of SA acted primarily as a substrate, whereas with higher intracellular concentrations the inhibitory effect became predominant, suppressing on the one hand yeast growth, on the other hand the metabolization of SA itself. In this action the undissociated SA molecules play the primary role which accounts for the pH-dependence of the effect. GSH influences the metabolization of SA, yeast growth on SA and growth inhibition by SA indirectly, through promoting the permeation of the SA molecules. Based on the experimental results, an explanation is offered for the dependence of the substrate or the inhibitory role of SA on certain factors and for the possible relationship between the two types of effects.

Sorbic acid (SA) is a selective fungistatic agent used primarily for food preservation (Deák, 1959). But decrease of the concentration of SA in, or even its "disappearance" from, the preserved product has been often noted, resulting in spoilage (Melnick and Luckmann, 1954; Melnick et al., 1954a, Costilow et al., 1955, 1957; Weaver et al., 1957; Alderton and Lewis, 1958). This phenomenon occurs chiefly in products in which the initial cell count is high or strong microbial action is involved in processing, as e.g. with pickles. Loss of 30—60%, sometimes even 100% of the added SA during the fermentation process of pickled cucumbers was noted also by one of us (Deák 1960a, b; 1963, 1964). As in the preserved products SA proved resistant to physical and chemical agents — heat, oxidation, plant tissue enzymes — its concentration decrease has been attributed to microorganisms (Boyd and Tarr, 1955; Melnick et al. 1954a; Schelhorn, 1954; Katona 1966). In fact, breakdown of SA by certain bacteria (York and Vaughn, 1954, 1955; Rehm et al., 1963, 1964) and various molds (Melnick et al., 1954b; Rehm and Lukas, 1963a; Lukas, 1964a, b) has been demonstrated experimentally. Nevertheless, evidence has been lacking to such activity of lactobacilli and yeasts, though

they were used both in pure culture and at high cell counts (COSTILOW et al.. 1955; REHM, 1967; REHM et al., 1964).

Studies in this laboratory on the effect of SA on growth and carbohydrate metabolism of yeasts have repeatedly shown concentration decrease of SA and consequent cessation of its growth inhibitory action in yeast cultures grown under aerobic conditions (DEÁK, 1967, 1969; DEÁK and NOVÁK, 1966, 1968, 1970; DEÁK and TÜSKE, 1967; DEÁK et al., 1970). Of the yeasts examined the phenomenon occurred with *Candida claussenii*, *C. pseudotropicalis*, *C. utilis*, *Procandida albicans* and *Pc. tropicalis*, while it did not occur in cultures of *Saccharomyces cerevisiae*, *S. carlsbergensis*, *C. beverwijkii*, and *C. krusei*.

Detailed examinations for the cause of the concentration decrease of SA disclosed that certain yeast species were capable of assimilating SA and of utilizing it for their growth.

1. Materials and methods

Sorbic acid was used in the form of potassium sorbate (Farbwerke Hoechst AG). Determinations of SA were performed as described earlier (DEÁK and NOVÁK, 1968; DEÁK *et al.*, 1970). The following yeast strains were examined: *Candida claussenii* 460-sect./1961, *C. krusei* 238/1964, *C. utilis* 287/1964, *Procandida albicans* 85/1957, *Pc. tropicalis* 302/1964, *Saccharomyces carlsbergensis* II/1966, *S. cerevisiae* CXCII/1967. The strains were maintained and propagated as described previously (NOVÁK and DEÁK, 1970).

Uptake of SA was examined by adding it at 0 time, in final concentrations specified later, to the cell suspension incubated under aerobic conditions, viz. aerated in a reciprocal shaker. Samples taken at predetermined time intervals were passed through a membrane filter (Sartorius MF 50) of 0,45 μ pore diameter to separate the cells and the diluted filtrate was assayed for SA content. Cells on the membrane filter were washed twice with ice cold distilled water, resuspended in ice cold phosphate buffer of pH 8.0 and extracted for 60 minutes. SA content was then determined again in the extract.

The composition of the medium used for the assimilation experiments was (g/1000 ml) KH_2PO_4 1.0, $MgSO_4 \cdot 7\ H_2O$ 0.5, $(NH_4)_2SO_4$ 5.0, in distilled water. To this medium potassium sorbate was added as sole carbon and energy source, in final concentrations to be specified later in terms of SA. For measurements at different pHs, a growth medium of double strength was prepared, sterilized, and mixed at a ratio of 1 : 1 with double strength buffer solution sterilized by filtration through a Schott G5 glass filter pad. The phosphate buffer was prepared from 1/15 M Na_2HPO_4 and KH_2PO_4 stock solutions so as to obtain the desired pH; with pH levels below 5.5, 1/15 M phosphoric acid solution was added in appropriate quantity to the Na_2HPO_4 stock solution.

The adjusted pH values were checked by means of an electric pH-meter (Orion KTSZ).

Aerobic incubation was made at room temperature (22 ± 2 °C), with 5 ml medium placed in each T-shaped test tube, as described previously (DEÁK and NOVÁK, 1968). Yeast growth was assessed by turbidimetry, as reported in earlier papers (DEÁK and NOVÁK, 1968; NOVÁK and DEÁK, 1970).

2. Results

In view of the concentration decrease of SA in growing aerobic cultures of yeasts (DEÁK, 1967, 1969; DEÁK and NOVÁK, 1966, 1968, 1970; DEÁK and TÜSKE, 1967; DEÁK et al., 1970) it was examined whether this phenomenon would occur also in resting stationary cell suspensions. With the cells shaken in phosphate buffer, there was a measurable decrease in the concentration of SA (Table 1), the degree of which depended on the time and pH as well as on the density of the cell suspension. In cell-free controls the concentration of SA appeared stable within the pH-range tested.

Table 1

Decrease of sorbic acid concentration in cell suspensions of Procandida albicans *incubated in phosphate buffer under aerobic conditions*

Initial concentration of sorbic acid: 71 μg/ml. Concentration of phosphate buffers: 1/15 M. 100 ml. suspension shaken in 500 ml flasks at room temperature (23 °C)

Concentration of the suspension (mg dry weight/ml)	pH	Per cent concentration decrease of sorbic acid after				
		10	22	46	56	75
		hours				
8.1	3.0	0	0	4	8	8
	5.5	0	0	22	25	29
	8.0	12	58	89	94	97
16.2	3.0	0	8	10	10	14
	5.5	9	31	55	78	79
	8.0	27	52	100	100	100
32.4	3.0	0	4	7	12	12
	5.5	2	9	56	86	100
	8.0	100	100	100	100	100
Cell-free	3.0	−2	0	−4	1	−4
control	5.5	0	0	0	−1	−5
	8.0	0	3	1	−2	−2

The above concentration decrease was not fully explicable by the cellular uptake or accumulation of SA. Though with the lower levels of SA added, its cellular uptake resulted relatively rapidly in the fall of extracellular SA with simultaneous rise of intracellular SA (Table 2), the decrease of SA in the medium was, however, greater than its increase within the cell, resulting in a loss

Table 2

Cellular uptake and accumulation of sorbic acid

Procandida albicans cell suspension (19.5 mg dry weight/ml), in pH 3.0 phosphate buffer. Initial concentration of sorbic acid: 75 μg/ml. Total volume: 4 ml, aerobic incubation for 20 minutes

Time (minutes)	Sorbic acid level			
	Extracellular (S_0)		Intracellular (S_i)	
	μg	μg/ml	μg	μg/ml
0	300	75	0	0
20	49	12.2	82	525

Cellular uptake of SA as calculated from concentration decrease of SA in the external solution: 300—49 = 251 μg. Numerical difference between measured and computed intracellular SA amount (SA loss): 251—82 = 169 μg, viz. 56% of total sorbic acid added. Ratio of accumulation: $S_i/S_0 = 43$. Calculating the intracellular sorbic acid concentration, the intracellular fluid was taken as 200% of the measured cellular dry weight (KOTYK and KLEINZELLER, 1963).

of the total amount of SA. The loss of SA tended to increase with time until both the intra- and extracellular quantities were reduced to zero (Fig. 1.). With increasing pH diminished the intracellular accumulation of SA (Fig. 2). The amount of SA taken up by the cell increased at a lower rate than the SA concentration, thus the per cent uptake decreased (Fig. 2). Increase of the concentration of SA resulted also in reduction of the loss of SA, i.e. the difference between the SA amounts taken up and intracellularly found (Fig. 3).

The above findings, too, support the supposition that the loss of SA is due to its breakdown by yeast cells in a process depending on the pH of the medium and on the concentration of the added SA. To substantiate this theory, attempt was made to cultivate the examined yeast species on SA as sole carbon and energy source (Fig. 4).

Out of the yeast species in aerobic cultures of which the concentration decrease of SA had been noted earlier (DEÁK, 1967, 1969; DEÁK and NOVÁK, 1966, 1968, 1970; DEÁK and TÜSKE, 1967; DEÁK *et al.*, 1970), *C. claussenii*, *Pc. albicans* and *Pc. tropicalis* showed distinct growth ability in SA, while *C. utilis*, *C. krusei*, *S. carlsbergensis* and *S. cerevisiae* grew less readily. The former three species had a mean multiplication index (*M*)* of 2.19 which, though

M* = final cell density/initial cell density (NOVÁK and DEÁK, 1970).

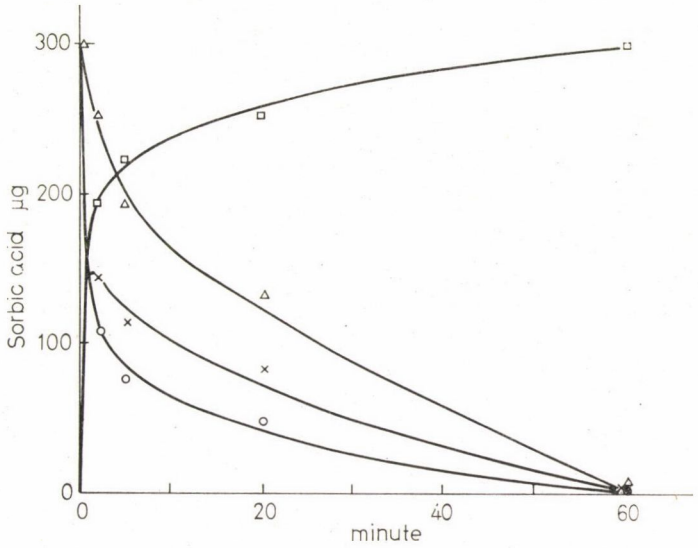

Fig. 1. Cellular uptake of sorbic as a function of time. *Procandida albicans* cell suspension (19.5 mg dry weight/ml) in phosphate buffer of pH 3.0, at 75 µg/ml initial concentration of sorbic acid, in 4 ml final volume, incubated under aerobic conditions at room temperature. *(1)* Sorbic acid content of incubation mixture ○ ——— ○; Cellular uptake of sorbic acid, as calculated from *(1)* □ ——— □; *(2)* Measured intracellular level of sorbic acid × ——— ×; Total sorbic acid content of the system *(1 + 2)* △ ——— △

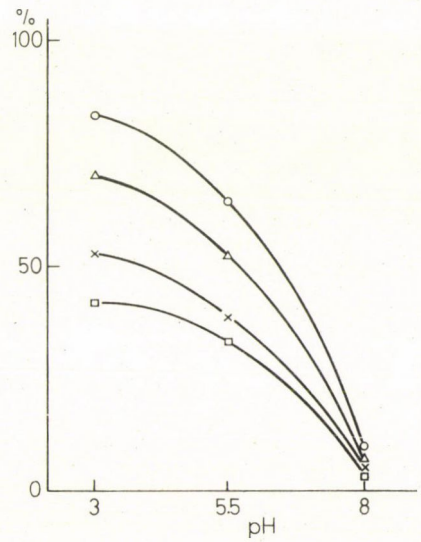

Fig. 2. Cellular uptake of sorbic acid as a function of concentration and pH. *Procandida albicans* cell suspension (12.2 mg dry weight/ml) in phosphate buffer, incubated in 2 ml final volume for 20 minutes under aerobic conditions. Initial sorbic acid concentration (µg/ml): 75 ○ ——— ○; 150 △ ——— △; 225 × ——— ×; 375 □ ——— □; Ordinate shows decrease of sorbic acid concentration, expressed as per cent of the initial level

20 times less than the corresponding index in glucose (DEÁK and NOVÁK, 1970), was significantly higher than the mean index of 1.35 assessed for the latter 4 species (L.S.D.$_{5\%}$ = 0.39). The rate of growth showed a correlation with the loss of SA in the medium (Table 3, Fig. 5), signifying the utilization of SA for yeast growth. The capacity to assimilate SA varied with the species, the most active being obviously *Pc. albicans*, which showed the most vigorous growth with a relatively low SA consumption (Table 3).

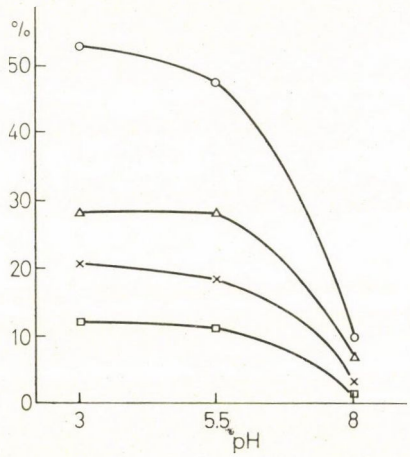

Fig. 3. Changes in sorbic acid loss as a function of sorbic acid concentration and pH. *Procandida albicans* cell suspension (12.2 mg dry weight/ml) in phosphate buffer, incubated in 2 ml final volume for 20 minutes under aerobic conditions. Initial sorbic acid concentrations (μg/ml): 75 ○ ——— ○; 150 △ ——— △; 225 × ——— ×; 375 □ ——— □; Ordinate shows the difference between measured and computed values of intracellular sorbic acid level (sorbic acid loss), expressed as per cent of the initial sorbic acid level

As can be seen from Fig. 4, SA assimilation was influenced also by the pH and the reduced glutathione (GSH) content of the medium. The effects of these factors and of the concentration of the inhibitor itself on the assimilation of SA were studied in more detail on *Pc. albicans*. The interaction of the three factors was remarkable (Fig. 6).

At pH 5.5, the growth rate decreased almost linearly with the elevation of the SA concentration; at pH 8.0, in contrast, it changed with the maximum curve and at pH 3.0, practically no growth occurred on SA medium, similarly to the SA-free control with M values in the range of 1. GSH added to the medium in equimolar concentration to the SA concentrations, substantially reduced the rate of multiplication at pH 5.5, while increased it at pH 8.0 on the SA substrate. Again a good correlation was demonstrated between the rate of multiplication and the quantity of SA utilized (assimilated) (Fig. 7).

Examination of the uptake of SA showed it to increase in the presence of GSH, with increasing concentration of the latter and of the pH (Fig. 8). Nevertheless, GSH-promoted increase of SA-uptake tended to decline with elevation of the concentration of SA in the medium (Table 4).

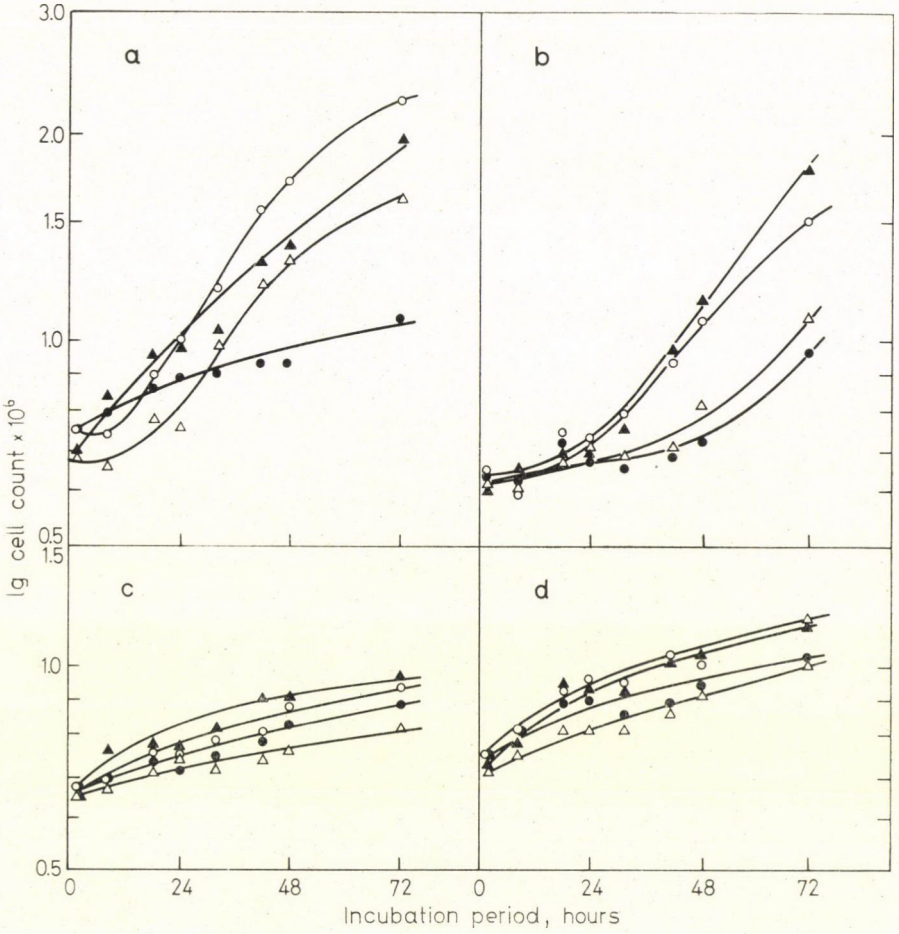

Fig. 4. Sorbic acid assimilation of yeasts. Aerobic cultures in synthetic medium, with an initial sorbic acid concentration of 190 μg/ml; reduced glutathione added at a ratio equimolar to the sorbic acid level (50 μg/ml). a. *Procandida albicans;* b. *Candida claussenii;* c. *Candida krusei;* d. *Saccharomyces cerevisiae.* ○ pH 5.5; ● pH 5.5 + GSH; △ pH 8.0; ▲ pH 8.0 + GSH

The change of the quantity of assimilated SA as a function of pH and SA-concentration was similar both in the presence or absence of GSH (Fig. 3). However in the presence of GSH the quantity of assimilated SA fell at pH levels 3.0 and 5.5 (except for the lowest SA concentration), while it increased at pH 8.0 (Table 5).

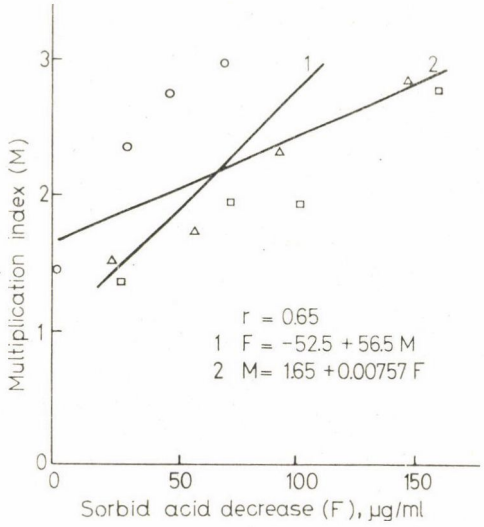

Fig. 5. Relationship between concentration decrease and assimilation of sorbic acid. Aerobic cultures in synthetic medium with sorbic acid added at an initial concentration of 190 µg/ml. Values of concentration decrease of sorbic acid and multiplication indices of yeast strains, as derived from Table 3., *Candida claussenii*, ○ *Procandida albicans*, *Procandida tropicalis*

Table 3

Yeast cell growth on sorbic acid and decrease of sorbic acid content during incubation

Aerobic cultures in synthetic medium, with initial sorbic acid concentration of 190 µg/ml; reduced glutathione added at equimolar concentration (500 µg/ml)

Species	Incubation system	Decrease of SA concentration in per cent after			Multiplication index at 72 hours
		24	48	72	
		hours of incubation			
Candida	pH 5.5	10.5	41.5	50.8	2.32
claussenii	pH 5.5 + GSH	0	4.5	14.8	1.51
	pH 8.0	2.8	20.0	30.7	1.72
	pH 8.0 + GSH	8.0	48.4	78.8	2.86
Procandida	pH 5.5	2.8	37.6	37.6	3.00
albicans	pH 5.5 + GSH	0	0	14.9	1.47
	pH 8.0	0	17.5	20.0	2.37
	pH 8.0 + GSH	1.6	20.0	25.6	2.77
Procandida	pH 5.5	10.4	36.0	40.0	1.97
tropicalis	pH 5.5 + GSH	10.4	12.0	16.0	1.37
	pH 8.0	5.6	20.0	56.0	1.97
	pH 8.0 + GSH	25.6	64.0	86.0	2.81

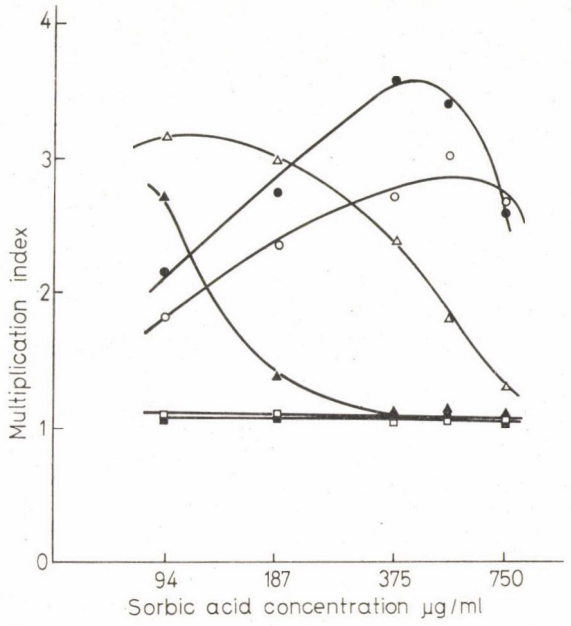

Fig. 6. Assimilation of sorbic acid as a function of concentration and pH, in the presence or absence of reduced glutathione. Aerobic cultures of *Procandida albicans* in synthetic medium. Reduced glutathione (GSH) added at concentrations equimolar to the indicated sorbic acid levels. □ pH 3.0; ■ pH 3.0 + GSH; △ pH 5.5; ▲pH 5.5 + GSH; ○ pH 8.0; ● pH 8.0 + GSH

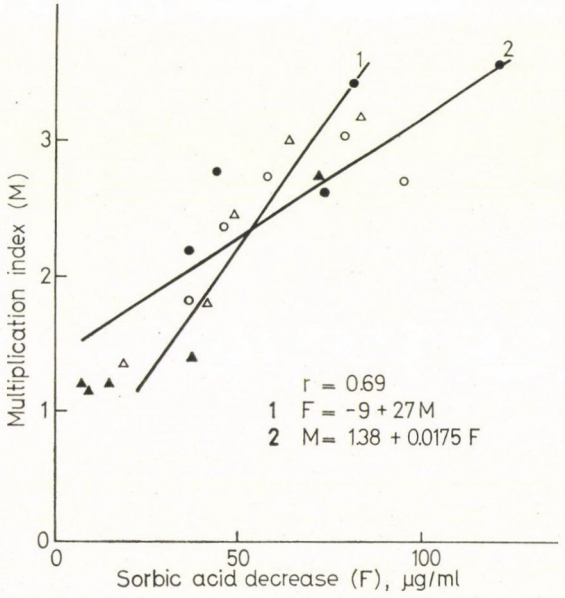

Fig. 7. Relationship between assimilation and concentration decrease of sorbic acid in the case of *Procandida albicans*. Aerobic cultures in synthetic medium. Values of multiplication index derived from Fig. 6. △ pH 5.5; ▲ pH 5.5 + GSH; ○ pH 8.0; ● pH 8.0 + GSH

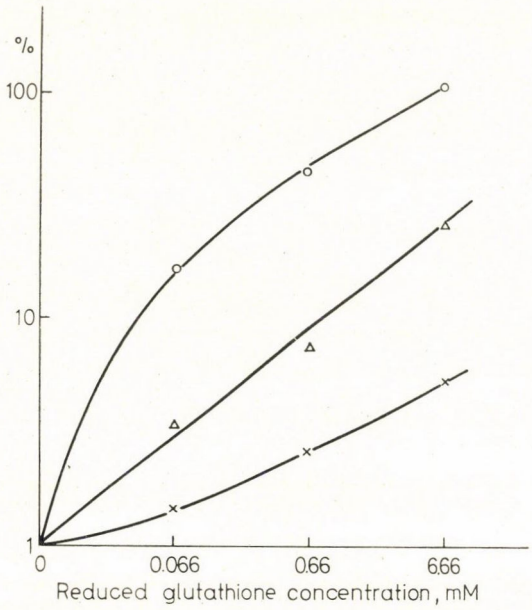

Fig. 8. Effect of reduced glutathione on cellular uptake of sorbic acid. *Procandida albicans* cell suspension (19.5 mg dry weight/ml) in phosphate buffer, incubated in 4 ml final volume for 20 minutes under aerobic conditions, with sorbic acid added at an initial concentration of 75 μg/ml (0.66 mM). The ordinate shows the increment of sorbic acid uptake as related to the control without added glutathione $\frac{(100 \times \text{GSH value} - \text{control})}{\text{control}}$
× ——— × pH 3.0; △ ——— △ pH 5.5; ○ ——— ○ pH 8.0

Table 4

Effect of reduced glutathione on cellular uptake of sorbic acid as a function of SA-concentration and pH

Procandida albicans cell suspension (12.2 mg dry weight/ml), in phosphate buffer, incubated in 2 ml final volume under aerobic conditions for 20 minutes. GSH added in tenfold molar concentration related to the SA level

Initial SA-level in medium μg	μg* sorbic acid taken up by the cells at								
	pH 3.0			pH 5.5			pH 8.0		
	—	GSH	%	—	GSH	%	—	GSH	%
300	251	265	106	193	246	127	31	66	213
600	420	430	102	315	376	119	44	81	184
900	474	496	105	348	384	110	52	87	167
1500	636	660	104	510	540	106	66	99	150

* Values calculated from decrease of extracellular SA level.

Table 5

Effect of reduced glutathione on the quantity of assimilated sorbic acid as a function of SA-concentration and pH

Procandida albicans cell suspension (12.2 mg dry weight/ml), in phosphate buffer, incubated in 2 ml final volume under aerobic conditions for 20 minutes. GSH added in tenfold molar concentration related to the SA level

Initial SA-level μg	μg* sorbic acid taken up by the cells at								
	pH 3.0			pH 5.5			pH 8.0		
	—	GSH	%	—	GSH	%	—	GSH	%
300	212	224	106	188	228	122	40	88	220
600	220	172	78	220	160	73	60	108	180
900	248	208	84	212	144	68	36	60	167
1500	240	224	93	228	168	74	16	28	175

* The values show the differences between the quantities of sorbic acid taken up by, Table 4), and found in fact in, the cells.

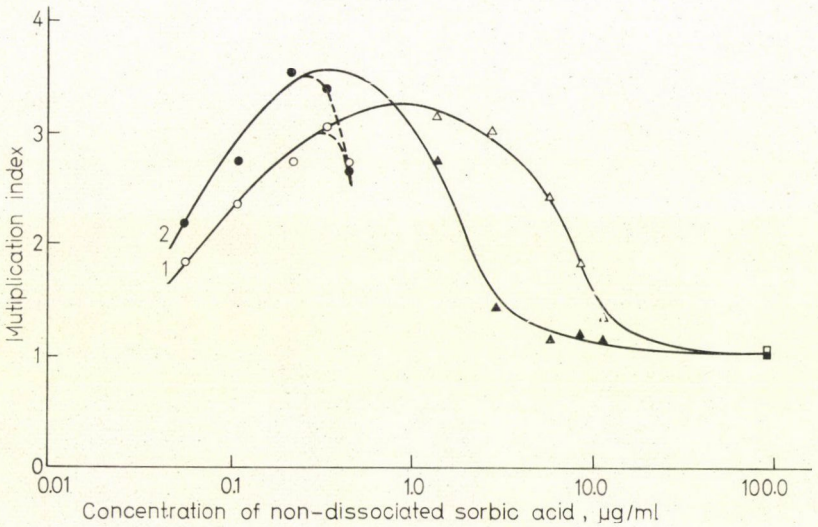

Fig. 9. Extent of yeast growth on sorbic acid as a function of concentration of undissociated sorbic acid molecules. Aerobic cultures of *Procandida albicans* in synthetic medium. Values from Fig. 6 : *1.* sorbic acid alone; *2.* sorbic acid + reduced glutathione at equimolar level. □ pH 3.0; ■ pH 3.0 + GSH; △ pH 5.5; ▲ pH 5.5 + GSH; ○ pH 8.0; ● pH 8.0 + GSH

Plotting the rate of multiplication versus quantities of undissociated SA at various pHs (Fig. 6), a maximum curve is obtained (Fig. 9). Obviously, elevation of the concentration of undissociated SA promoted multiplication up to a level of about 1 μg/ml, above which the inhibitory action of SA came

into display. The presence of GSH altered the rate of multiplication so as if the concentration of undissociated SA had been higher than actually, as established from growth values without GSH.

3. Discussion

The present data permit of the conclusion that certain yeast species — of those examined primarily *Pc. albicans*, *Pc. tropicalis* and *C. claussenii* — are capable of assimilating SA. Utilization of SA for yeast growth was suggested by the findings as follows: 1. intracellular SA concentration was less than the amount actually taken up by the cells from the medium (Table 2, Fig. 3); 2. yeasts were capable of growing on SA as sole carbon and energy source (Fig. 4); 3. a correlation was demonstrable between the extent of yeast multiplication and the concentration decrease of SA in the medium (Table 3, Figs 5 and 7). This capability of yeasts, first recognized by us, accounts for the occasional failures of SA-preservation of those products which, after the degradation of SA, would by nature favour yeast growth, such as dessert vines, concentrated fruit juices and other beverages. Explanation for the present findings on SA assimilation is offered below, with due consideration to the influences of inhibitor concentration, pH and the presence of GHS.

3.1. Degradation and assimilation of sorbic acid

In a certain concentration range, SA taken up by yeast cells under aerobic conditions plays the role of a substrate, while above that concentration range its inhibitory effect comes into display. Within the cells of the yeast species capable of degrading SA, the inhibitor undergoes metabolization until the rate of SA uptake and the degradation process are in equilibrium. If, for example, owing to the elevation of SA concentration in the medium the cells take up more SA than they are able to metabolize, the inhibitory action of SA becomes predominant, interfering with the decomposition of SA itself. With elevation of the concentration of SA the loss of total SA content of the system decreases (Fig. 3), resulting in the intracellular accumulation of so much SA as to interfere with multiplication (Fig. 6). The influence of further factors (pH, GSH) also comes into effect through quantitative alteration of SA-uptake by the cells.

3.2. Uptake of sorbic acid

In the cellular context, the undissociated SA is of primary importance whether it plays the role of the substrate or of the inhibitor. Evidence has been presented by many authors (Schelhorn, 1954; Nomoto et al., 1955; Bell et al., 1959; Azukas et al., 1961; Oka, 1960a, b, 1962; Rehm and Lukas,

1963b) that the antimicrobial action of SA is determined by the proportion of its undissociated molecules, to which the cell membrane is being more permeable and the proportion of which depends on the pH. The results of this study confirmed the above statement (Fig. 2) in that the uptake of SA was found to decrease with the rise of the pH, in accordance with the diminution of the proportion of undissociated molecules. The driving force of SA-uptake is obviously the difference between the intra- and extracellular pHs, according to the triple equilibrium

$$SA^- \rightleftharpoons SA \rightleftharpoons SA \rightleftharpoons SA^-$$

cell membrane

Conway and Downey (1950) and Kotyk (1963) give the intracellular pH as 5.8. Accordingly, at extracellular pHs lower than that, the total (dissociated and undissociated) intracellular concentration of SA may be higher than its extracellular concentration, even in the absence of intracellular adsorption. On the other hand, solutions with pHs higher than 5.8 may be utilized for the extraction of intracellular SA. This has been confirmed by the present experiments (Table 2, Fig. 3), consistently with the literary data (OKA, 1960a, b; 1962). Nevertheless, the circumstance that SA entered the cells also at pH 8.0 in a greater degree than accounted for by the pumping effect due to the metabolization of intracellular SA (Tables 4 and 5) suggests that to a certain extent also factors other than the ratio of SA : SA^- as determined by the difference between extra- and intracellular pH may play a role in the uptake of SA. But there is as yet no evidence whether the entry of SA into yeast cells is related to intracellular adsorption of SA, dissociated or undissociated, as supposed by OKA (1960a, b, 1962), or to the low degree of permeation of the dissociated molecules as interpreted by Rehm and Lukas (1963b). A third alternative would be the existence of a transport system which would actively promote uptake and accumulation of SA even at low concentrations of undissociated molecules.

Independently of the above considerations, the present results of SA assimilation experiments may be interpreted also by supposing that increase of the intracellular level of undissociated SA, viz. of the substrate to be assimilated, would result first in rise of the rate of multiplication which, after having reached a given maximum, will decrease and finally stop, owing to the inhibitory effect of the increasing level of undissociated SA (Figs 3 and 6).

3.3. Effect of reduced glutathione

The opposite actions of GSH at pH 5.5 and pH 8.0 in the SA assimilation experiments appear to have been due to the indirect influence of GSH by

promoting the permeation of the undissociated SA molecules. Therefore, at pH 8.0 at which the concentration of undissociated SA was low, GSH, through the promotion of permeation, was able to increase the intracellular level of assimilable substrate, viz. to enhance growth ability on SA. In contrast, at pH 5.5 at which the proportion of the undissociated SA utilizable as substrate was greater, GSH enhanced the rate of penetration to such an extent that upset the equilibrium between the quantities of SA taken up by the cells and decomposed within them, so that the inhibitory action of SA became predominant, effecting decrease and finally cessation of SA assimilation and yeast growth on SA (Fig. 6).

Experiments on uptake of SA served direct proof for the SA penetration enhancing effect of GSH (Fig. 8, Table 4). Consistently with the above hypothesis at higher concentrations of SA, GSH acted indirectly toward the reduction of the metabolism-related SA loss (Table 5), to the analogy of the direct effect of elevated SA level (Fig. 3). In accordance with the findings on SA assimilation, the presence of GSH acted at pH 8.0 toward the increase, whereas at pHs 3.0 and 5.5 toward the decrease, of metabolism-related SA loss (Table 5).

The SA-uptake enhancing action of GSH perhaps develops through influencing the SH groups of structural or functional proteins on the external surface of the cell. It is known that the SH groups of the cell membrane play an important role in the cation transport of red blood cells (SUTHERLAND et al., 1967) and that the rearrangement of these groups under the influence of oxidated glutathione or cystine involves inhibiton of K—Na ATPases (DICK et al., 1967). The SH groups play an important role also in the permease systems of bacterial cells (KEPES, 1969). Nevertheless, it remains to be clarified whether the SH groups of the cell membrane and the influence of GSH on them, are of a similar significance in the uptake of other types of compounds, as e.g. SA.

3.4. Relationship between inhibitory action and assimilation of sorbic acid

It follows from the above considerations that the extent of assimilation of, or inhibition by SA would change as a function of undissociated SA level in the form of a maximum curve. This type of curve was actually obtained by plotting out growth results on SA versus concentration of undissociated SA calculated from the concentration of added SA and from the pH (Fig. 9.) The peak of the curve was at about 1 μg/ml undissociated SA concentration which seems to be the limit above which the action of SA becomes inhibitory. But this value applies to the extracellular SA level; the intracellular one may be higher than that owing to accumulation of SA in the yeast cells (Table 2; OKA 1960a, b, 1962). It should be noted that the ascending part of the curve, derived from measured and computed values at pH 8.0, is reclining. This is perhaps due to the circumstance that at pH 8.0 and at higher levels

of SA, sorbate anions are present in the system in such an amount that their effect is no longer negligible. The inhibitory action of sorbate anions has been shown to be only 30—100 times lower than that of undissociated SA (REHM and LUKAS, 1963b). Plotting the values measured in the presence of GSH and SA in the same manner as above, the curve obtained (Fig. 9) showed shift of the peak toward the lower level of undissociated SA. Supposing that the intracellular SA concentrations corresponding to the identical growth rates were identical also, the indirect conclusion can be drawn again that GSH promoted the permeation of a substantial amount of SA even at lower levels of the latter in the medium. The same has been confirmed by the experiments on direct SA-uptake.

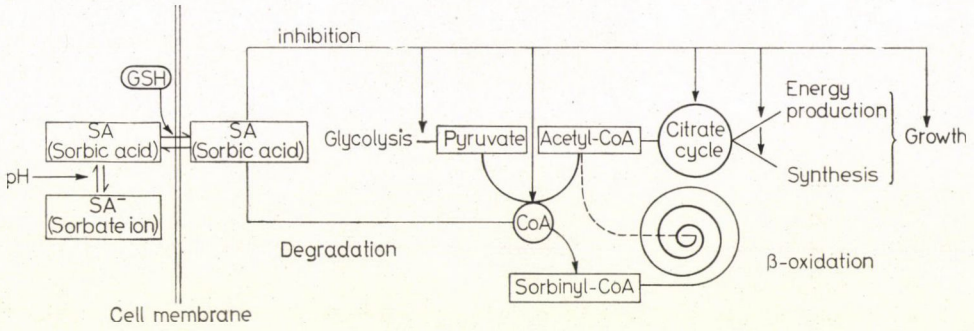

Fig. 10. Schematic presentation of probable relationship between assimilation and growth inhibitory action of sorbic acid. See explanation in text

In view of the relevant literary data it seems highly possible that the degradation of SA in the microbial cell takes place in the same manner as in the animal cell, viz. following the course of the so-called β-oxidation of fatty acids (MELNICK et al., 1954b, REHM, 1967). A somewhat dissimilar pathway is suggested by the presence of methylketone in addition to the CO_2 and H_2O end products (LUKAS, 1964a, b); appearance of methylketone may be, however, due also to inhibition of fatty acid metabolism. Details of the mechanism of action of SA inhibition being beyond the scope of this report, the reader is referred to a relevant publication by DEÁK (1969). Mention is, nevertheless, made of literary data (PALLERONI and PRITZ, 1960; HARADA et al., 1968) explaining SA inhibition by competition for the coenzyme A. In agreement with this interpretation, also ANDERSON's (1963) hypothesis seems highly possible that the yeast species being unable to decompose SA are lacking the respective fatty acid activating enzyme required for the formation of the sorbinyl-coenzyme A. By virtue of its central role in anabolic and catabolic processes, coenzyme A may represent the link through which either the substrate or inhibitory properties of SA come into display. The relationship between the two alternatives is illustrated in Fig. 10. Fatty acids are known to participate in

the β-oxidation cycle in the form of coenzyme A derivatives. SA, in the form of sorbinyl-coenzyme A, may become metabolized in the course of this cycle and consequently become assimilated as a substrate. The larger amount of SA entering the cells at higher external levels of SA, at lower pHs or in the presence of GSH may bind the total coenzyme A reserve of the cell, thereby stopping on the one hand the decomposition of SA itself, on the other hand suppressing the metabolic processes of the cell resulting in, as a final outcome, the inhibition of cellular growth.

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KINETICS OF AMYLOLYSIS

I.—FURTHER STUDIES ON AUTO-INHIBITION OF BETA-AMYLOLYSIS

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In the course of the beta-amylolysis of amyloses not only reactive, but also inactive complexes of enzyme and substrate may form. Though the cleavage of bonds is brought about by the reactive complexes, the follow-up of inactive complex formation is essential for the understanding of the kinetics of beta-amylolysis, because such complexes withhold part of the enzyme from the main process. To find the proper orientation among the controversial literary data, in this laboratory the virtual Michaelis constants and maximal velocities were determined for amylodextrines and amyloses at different degrees of polymerisation. (DP = 5 and 6; DP = 24, 48 and 250, respectively). Increase of the degree of polymerization from 5 to 250 resulted in an about 36fold reduction of the Michaelis constants. The affinity of beta-amylase was 6.85 times greater to the non-reducing glucose terminal units of the substrates than to the intrinsic glucose units of the molecule. Thus the probability of the formation of an inactive complex appeared identical for each coil of the helix.

The same increment of the degree of polymerisation reduced the maximal velocities only 3.5 times. Reduction of the virtual Michaelis constants was actually due to auto-inhibition by the internal segments of the amylose molecule, but in the reduction of the maximal velocity also other factors such as change of diffusion velocity, associations, etc. played a role. This was confirmed by decomposition of the substrate of "infinite" molecular weight, containing precisely 250 glucose units.

The amylolytic degradation of starch is a significant physiological process, at the same time it is relevant also in the industrial context, be it for the utilization of plant enzymes (e.g. mashing in brewery, or production of spirits etc.) or industrially produced microbial amylase preparations (liquefaction of starch, etc.). The products of various enzymatic processes, chemical and physical processes affecting the functions of amylolytic enzymes and the most important functional groups which may play a role in the catalytic activity of amylases are fairly well known. The precise mechanism of the catalysis is, nevertheless, poorly understood and explanations are in most part hypothetical (KOSHLAND et al., 1962; MAYER and LARNER, 1959; FRENCH, 1957; FISCHER and STEIN, 1960; NAKAMURA, 1962).

Kinetic studies of amylolysis in this laboratory were aimed at presenting experimental data in support of one or another of the available theories and to offer an explanation for the extraordinary reaction velocity of enzymic glycolysis which is 10^9—10^{12} times faster than protonic catalysis (THOMA, 1968; HOLLÓ and LÁSZLÓ, 1969).

Examining the inhibitory effect of substrates of various molecular weights and of cycloamylose in beta-amylolysis, THOMA and KOSHLAND (1960) demonstrated that also the internal segments of the amylose chain inhibited catalysis, as they were able to form with the enzyme complexes which did not produce cleavage of bonds and thus were inactive. These authors believed that the decrease of the Michaelis constant with the increase of the substrate chain length attested to the phenomenon of autoinhibition. A similar decrease was noted by PFANNEMÜLLER (1968) in the case of potato phosphorylase. But for barley amylose samples degraded mechanically in a colloid mill, RICHTER (1964) found a decrease of K_m only in the range of DP (degree of polymerization) = 15—34; above that range the Michaelis constant (K_m) remained unchanged. THOMA and KOSHLAND (1960) did not report the changes of the maximal velocities. Thus the proof presented by them for auto-inhibition on the basis of their own findings and data by others is not unequivocal. On the other hand, measurements in this laboratory and the K_m values described for amyloglucosidase (ONO, 1960) have confirmed the fact of auto-inhibition.

Experiments were, therefore, performed to determine whether the lower initial velocity found with amyloses of rising degrees of polymerisation was due to auto-inhibition only or also to other factors. This paper is a report of the experimental results obtained with beta-amylase from sweet potato.

1. Materials and methods

Amylose fractions of different degrees of polymerisation were extracted from with acid partially hydrolysed wheat amylose, by multiple alcoholic fractionation. The molecular weight distribution of the products was determined by iodine titration (RICHTER, 1966) and they were stored in the form of buthanol complexes.

Maltooligosaccharides were obtained by alpha-amylase degradation of wheat amylose and separation on active carbon—celite chromatographic column. The products were homogeneous, as ascertained by paper chromatography. Crystalline beta amylase from sweet potatoes was procured from the firm SIGMA.

Amylose was coupled with inactive molecules as follows: the terminal reducing groups of amylose were oxidized to carboxyl groups by means of hypoiodide and esterified with p-nitrophenol in dimethylsulfoxide in the presence of dicyclohexylcarbonic diimide dehydrant. The active ester thus obtained was coupled with heat-denaturated insoluble bovine albumin in aqueous medium. The amount of amylose which did not enter into the reaction was removed by washing with water. The molecular weight of the coupled product was practically "infinite".

Table 1

Decomposition by beta-amylase of DP = 250 amylose bound to insoluble albumin

Enzyme concentration: 9.5×10^{-9} M/litre. Temperature: 30 °C. pH = 4.8 (0.02 M acetate buffer)

Concentration of substrate (M/litre) $\times 10^5$	Quantity of maltose formed (mg/litre × 30 min.)	Initial reaction velocity (M/litre × min.) $\times 10^6$
1.75	23	3.10
3.50	37	3.98
7.00	103	4.38

Table 2

Initial reaction velocities measured at the degradation of DP = 250 amylose by beta-amylase

Enzyme concentration: 9.5×10^{-10} (M/litre) Conditions of reaction: same as in Table 1

Concentration of substrate (M/litre $\times 10^5$)	Quantity of maltose formed (mg/litre × 10 min.)	Initial velocity (M/litre × min.) $\times 10^5$
5.3	72	2.02
4.25	63	1.81
3.18	58.5	1.75
2.66	55	1.69
2.12	45.5	1.307
7.80	88	2.60
5.53	75	2.15
4.00	64.5	1.89
2.77	62	1.77
2.02	48	1.36
4.78	67	2.00
3.83	72	2.10
2.86	63	1.86
1.92	37.5	1.215
5.03	67	1.92
4.02	59.5	1.75
3.05	53	1.487
2.52	60.2	1.71
2.02	44.5	1.252

Enzymic degradations were carried out under optimal conditions (at 30 °C, pH = 4.8, in 0.02 M acetate buffer). The quantities of maltose which formed in 10 minutes were determined by Somogyi and Nelson's procedure

Table 3

Initial reaction velocities measured at the degradation of DP = 48 amylose by beta-amylase

Conditions of reaction: same as in Table 2

Concentration of substrate (M/liter)10^5	Quantity of maltose formed (mg/litre × 10 min.)	Initial reaction velocity (M/litre × min.) × 10^5
18.6	116	3.255
14.0	106	2.885
9.3	95	2.626
4.64	61	1.648
3.72	51	1.395
15.6	132	3.740
11.7	104	2.940
7.8	85	2.290
3.91	52.2	1.340
3.12	42.6	1.150
26.3	157	4.290
21.0	113	3.190
15.8	102.5	2.890
10.5	85.2	2.380
5.25	58.2	1.570
21.9	125	3.510
17.6	140	3.850
13.2	116.5	3.190
8.75	90.5	2.460
4.4	54.7	1.460
19.9	133	3.740
14.9	109.5	3.060
9.9	87.5	2.400
5.0	54	1.470
4.0	53	1.410

(HODGE and HOFREITER, 1962). The acitivity of the beta-amylase preparation was checked with the dinitrosalicylic acid procedure.

1.1. Decomposition of DP = 250 amylose linked with insoluble albumin: Amylose linked with 43.7, 87.5 or 175 mg of carrier substance was hydrolysed in a heterogeneous medium, at 600. rpm., 30 °C and pH 4.8. Concentrations of amylose in the three reaction mixtures were calculated as 1.75×10^{-5}, 3.5×10^{-5} and 7×10^{-5} M/litre, respectively. The enzyme concentration was 9.5×10^{-9}. Maltose contents determined in samples taken at different points of time and the velocities calculated from them are shown in Table 1.

Table 4

Initial reaction velocities measured at the degradation of DP = 24 amylose by beta-amylase

Conditions of reaction: same as in Table 2

Concentration of substrate (M/litre × 10^4)	Quantity of maltose formed (mg/litre × 10 min.)	Initial reaction velocity (M/litre × min.) × 10^5
3.2	195	5.19
2.56	126.5	3.50
1.92	119.5	3.21
1.6	109.5	2.93
1.28	109.5	2.84
3.2	165	4.495
2.56	154	4.125
1.92	137	3.64
1.6	103.5	2.75
1.28	89	2.38
2.18	130.5	3.60
1.75	126	3.25
1.31	123	3.21
1.08	87	2.30
0.87	83	2.13
2.18	121	3.36
1.75	104	2.92
1.31	97.5	2.56
1.08	96.5	2.51
0.87	81.5	2.08

1.2. Enzymic degradation of DP = 250 amylose: Amyloses of different degrees of polymerisation were used in the form of buthanol complexes, thus the amylose contents of the solutions could not be precisely determined until the removal of buthanol by distillation, using the phenol-sulfuric acid method (DUBOIS et al., 1956). Thus the amylose concentrations

Table 5

Initial reaction velocities measured at the degradation of maltohexaose by beta-amylase

Conditions of reaction: same as in Table 2

Concentration of substrate (M/litre) $\times 10^5$	Quantity of maltose formed (mg/litre $\times$ 10 min.)	Initial reaction velocity (M/litre $\times$ min.) $\times 10^6$
14.3	4.10	10.30
	4.28	11.80
	3.42	9.55
11.4	4.35	9.35
	3.25	8.64
	2.74	8.17
8.6	2.90	7.35
	2.80	7.10
	2.70	6.12
7.15	1.74	5.03
	1.67	4.90
	1.98	5.92
5.72	1.54	4.55
	1.36	4.10
	1.92	5.70

determined in replicates were dissimilar. Values for the initial velocities of maltose measured during 10-minutes reaction time under the above conditions, are shown in Table 2. The concentration of the enzyme was 9.5×10^{-10} M/litre.

1.3. Hydrolysis of DP = 48 and DP = 24 amylose: data found under conditions similar to those specified above are shown in Tables 3 and 4. The enzyme concentration was 9.5×10^{-10} M/litre.

1.4. Enzymic decomposition of maltooligosaccharides: Beside amyloses of different molecular weights maltooligosaccharides were also tested for the values required to confirm auto-inhibition and to calculate the constant for the actual velocity of product formation. Results for maltohexaose and maltopentaose are shown in Tables 5 and 6.

Table 6

Initial reaction velocities measured at the degradation of maltopentaose by beta-amylase

Conditions of reaction: same as in Table 2

Concentration of substrate (M/litre) $\times 10^4$	Quantity of maltose formed (mg/litre $\times$ 10 min.)	Initial reaction velocity (M/litre $\times$ min.) $\times 10^6$
2.9	4.90	13.85
	7.20	20.52
2.32	6.85	19.75
	4.04	11.79
1.74	4.68	13.56
	3.18	9.27
1.1	2.43	7.49
	2.26	6.58
0.58	1.43	4.18
	1.33	3.83

2. Results

Virtual Michaelis constants and maximal velocities were calculated from data for six substrates of different molecular weights, using the method of Lineweaver and Burk. Owing to inaccuracies of distribution, as well as liability to association and intricate steric structure of the amylose molecules, many points of measurement are necessary for the appropriately precise

determination of the above constants. Straight lines for the individual substrates were constructed by the principle of the least squares of error calculated by computer technique (Hewlett Packard 9100 A). The values of regression were the following:

DP = 250: 0.98 0.98 0.93 0.85
DP = 48: 0.99 0.99 0.99 0.99
DP = 24: 0.98 0.89 0.98 0.94
DP = 6: 0.97 0.97 0.99
DP = 5: 0.98 0.98 0.99

The mean values of the individual kinetic constants and standard deviations in the individual series were also determined. Comparison of the means with the results of a correlation analysis in which all data were considered showed them to be in good agreement. The virtual Michaelis constants obtained for the six different substrates and the maximum velocities, together with standard errors of the mean are shown in Table 7.

As can be seen from Table 7, increase of the degree of polymerisation of the substrate from DP = 5 to DP = 250 resulted in about 36fold decrease of the Michaelis constant, while the decrease of the reaction velocity was only

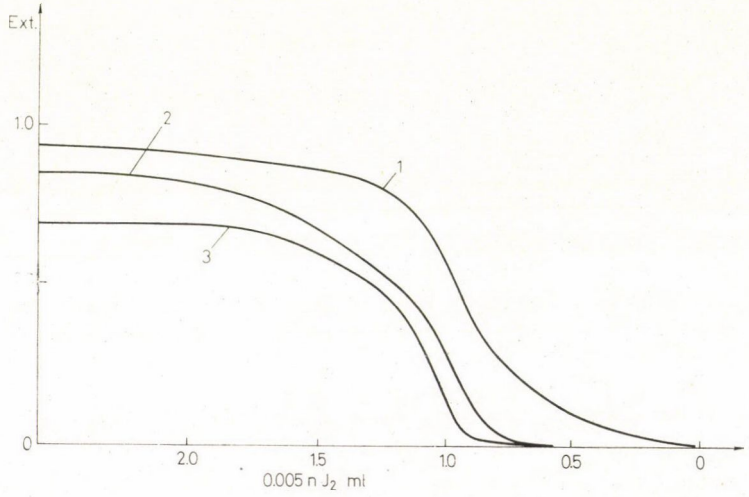

Fig. 1. Determination by photometric iodine titration (RICHTER, 1964) of molecular weight distribution of DP = 250 (1), DP = 48 (2) and DP = 24 (3) amylose. Concentration of amylose: 0.2 mg/100 ml; Iodine concentration: 0.005 N; Layer thickness: 3.4 cm (length of cuvette)

about 3fold. But the Lineweaver-Burk curves do not allow the separation of the intrinsic "inhibitor concentration", responsible for the auto-inhibition, from the concentration of the substrate. A more accurate estimation of auto-inhibition is obtained if instead of the molar concentrations of oligomeric and polymeric substrates the reciprocal concentration of all glucose units

Table 7

Virtual Michaelis constants and maximal velocities determined with different substrates

(± values represent the errors of the mean)

Degree of polymerisation of the substrate	$K_m \times 10^5$ (M/litre)	$V_{max} \times 10^4$ (M/litre × min.)
250*	1.0	52.0
250	2.78±0.14	3.14±0.13
48	13.4 ±0.3	5.95±0.09
24	22.2 ±0.6	7.25±1.3
6	105.0 ±5	8.62±0.1
5	125.0 ±6	8.70±0.1

* Sample bound to insoluble albumin

taking part in the formation of the enzyme-substrate complex are plotted versus the reciprocal values of the initial velocity (Fig. 2). In this presentation the maximal velocities are, naturally, identical to those shown in Table 7, but the virtual Michaelis constants will be dissimilar (Table 8).

Table 8

Virtual Michaelis constants for substrate concentrations expressed in terms of glucose units

Degree of polymerisation of substrate	$K'_m \times 10^3$ (AGU M/litre)
250	6.75
48	6.42
24	6.37
6	6.30
5	6.25

AGU = anhydro-glucose unit

As can be seen from Table 8, the virtual Michaelis constants decreased only very slightly with substrates of different degrees of polymerisation, which again confirms the decisive role of auto-inhibition. But slight changes of the K_m and alterations of maximal velocity in connection with inactive complex

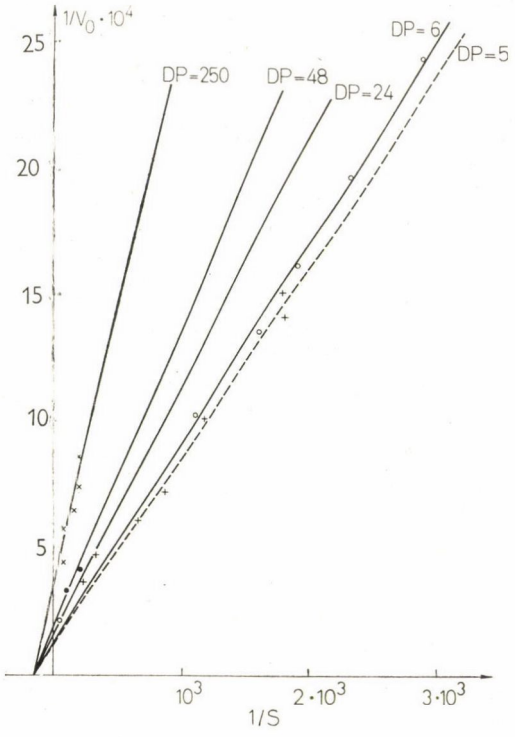

Fig. 2. Lineweaver—Burk-type diagram with data calculated as concentration of glucose units. Abscissa: 1/glucose unit concentration in litre/M dimension. Ordinate: $1/v_0$-, reciprocal value of initial reaction velocity in the dimension: M/litre × min. The figures designating the lines correspond to the degrees of polymerisation

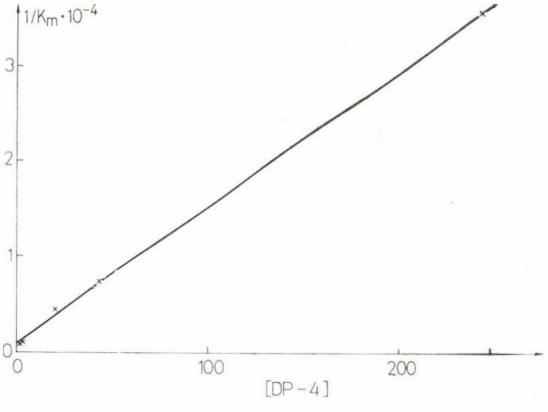

Fig. 3. Changes of affinity to beta-amylase at different degrees of polymerisation of the substrate. Abscissa: numerical values for degree of polymerisation (DP)-4. Ordinate: reciprocal value of the virtual Michaelis constant

formation stress the necessity of the consideration of factors other than the above. Thus, in addition to the relation $1/K_m$—DP (Fig. 3) presented in support of auto-inhibition, also the relation $1/V_{max}$—DP should be investigated in more detail. Using the numerical values of the two variables, a more demonstrative diagram is obtained if the relation V_{max}—1/molecular weight is represented graphically. In this case also the data of the carrier-bound amylose can be taken into consideration (Fig. 4).

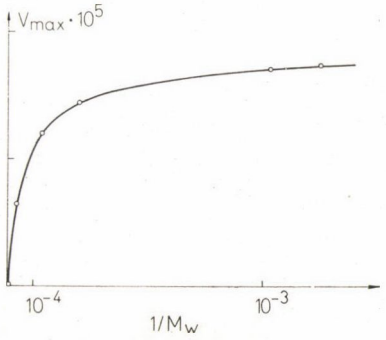

Fig. 4. Changes in maximal velocity as a function of the reciprocal value of the substrate's molecular weight. Abscissa: reciprocal value of molecular weight. Ordinate: maximal reaction velocity

3. Conclusions

With amylose substrates, inaccuracies in the weighing of the material (irrespective of the solvent being buthanol complex, alkali or other dispersoid medium), the intricate steric structure of the amylose molecule, various transformations of the dynamic equilibrium of the random coil as well as the liability of the amylose molecule to association render the precise determination of kinetic constants for enzymic decomposition very difficult. However, certain conclusions can be drawn with fair accuracy from some serial assays, in replicates wether evaluated by regression analysis of single series or by correlation analysis of all the series.

As shown in Table 3, with the beta-amylase substrate of the lowest degree of polymerisation no abnormal decrease of the reaction velocity was observed, but the extrapolated Michaelis constant was 1.0×10^{-3} M/litre. This was 36 times higher than the value calculated for DP = 250 amylose, suggesting a 36 times higher affinity of beta-amylase to DP = 250 amylose than to maltotetraose, owing to inactive enzyme binding by the intrinsic segments of amylose. The extrapolated value is in good agreement with the 0.89×10^{-3} M/litre value determined by THOMA and KOSHLAND (1960), with sweet potato beta-amylase at 25 °C. The K_i value calculated from the

slope of the regression line was 6.85×10^{-3} M/litre, which was the inhibition constant related to the intrinsic glucose units of the amylose molecule. This implies that with the amylose polymerized to degree N, (N—4)/6.85 inactive enzyme-substrate complexes and one active complex may form, the total number of complexes being 1 + (N—4)/6.85. The quantity of enzyme bound in active and inactive substrate complexes naturally depends on the concentration of the substrate, complying with the theory of Michaelis, and at saturated substrate levels the total quantity of beta-amylase will be found in the form of active enzyme-substrate complex. The affinity of one terminal group is identical to that of 6.85 intrinsic glucose units, which corresponds to the average number of coils in the helix.

With the substrates examined, the number of inactive bonds for one active enzyme-substrate complex was as follows:

at DP = 250	36
at DP = 48	6.4
at DP = 24	2.9
at DP = 6	0.29
at DP = 5	0.145
at DP = 4	0

The horizontal intercept of the Lineweaver — Burk diagram for the substrate (inhibitor) level calculated in glucose units (Fig. 2) was about identical with the inhibitor constant for one glucose unit ($6.25—6.75 \times 10^{-3}$ M/litre).

The dependence of the maximal velocity on the molecular weight-substrate correlation suggests that beyond the auto-inhibition also other factors (diffusion velocity, viscosity, steric structure, associability, etc. of the substrate) play a role. But further studies are required to obtain experimental evidence.

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DISPERSOID-ANALYTICAL STUDIES ON COMMINUTION OF FOOD SUSPENSIONS

I. EXPERIMENTS WITH ULTRASONIC COMMINUTORS

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(Received October 19, 1970)

Suspensions of quince, plum, apricot and tomato fibres were disintegrated in two types of ultrasonic comminutors, the Minisonic and the MSE ultrasonic disintegrator.

In both types of apparatus, the specimens were exposed to comminution for various periods and the suspensions thus obtained were subjected to dispersoid-analytical examination, using the sedimentation technique elaborated by the author. The granulometric data and the corresponding standard deviations were calculated for each suspension and tabulated together with the data of mathematical-statistical analysis.

Particle size distribution in the system was characterized numerically by Trask's parameters and represented diagrammatically as a function of time of exposure in the comminutor.

Experiments in the Minisonic apparatus have shown that the maximal reduction of particle size took place in the first phase of comminution and that the originally roughly dispersed suspensions underwent comminution to a greater degree than the finely distributed ones.

Similar conclusions were drawn from the experiments in the MSE ultrasonic disintegrator.

The maximal comminuting effects of the two comminutors tested were characterized numerically by C_{25}, C_{50} and C_{75} values, which represented the quotients of the Trask parameters Q_1, Md, and Q_3, respectively, for the suspension in untreated and maximally comminuted state.

Determination of the above C (comminution) values has unequivocally shown that the Minisonic apparatus had the greater comminuting efficiency.

The preparation of many food products involves the comminution of plant tissues. The dispersoid analytical aspects of comminution have been studied with various types of disintegrators, of which only a few are mentioned here on the basis of literary data.

ROSENBAUM and SITNIKOV (1963) have devised a steam-jet homogenizer for the comminution of food products. The substance to be disintegrated is placed into a flow of steam or compressed air, which carries it at a supersonic speed through a sieve, whereby the tissues become disrupted. The device was tested with tomato and apple juices and it was found to be suitable for their processing.

REES (1967) describes two devices, one of which is a colloid mill, deriving high energy from vortex motion and shear flow. The energy is produced by a rotor, rotated at high speed close to a stator. There are two variants of this apparatus: in one of them the substance to be comminuted flows in the direction of the rotor's centrifugal power, in the other in the opposite direction.

The other device is a homogenizer, reported by REES to be suitable for the homogenization of apricot and apple juices.

Ultrasonic research has made a great progress during recent years and as a result economic methods have been elaborated for the utilization of ultrasonic techniques in the preparation of suspensions and emulsions.

According to ROSE (1954) comminution by ultrasound produces a particle size of 1 μm, enabling the preparation of stable emulsions or suspensions without the addition of a stabilizer; which is, in the case of food products, a great advantage. ROSE describes a device which produces ultrasound by a vibratory wedge in the liquid flow. The cavitation arising around the vibratory wedge extremely accelerates molecular movement in the medium passing through the wedge, resulting in local "explosions" which produce pressures in the range of 2000 kp/cm^2.

ZOLOTOVA (1965) reports ultrasonic comminution of fibres in tangerine, peach and tomato juices, using a quartz vibrator at a frequency of 1 MHz. The peach juice treated for 1 minute did not separate into two phases in 24 hours, while the untreated control separated in 1.5—2 hours. Tomato juice treated for two minutes took double the time to sediment than the untreated juice. Less promising results were obtained with tangerine juice.

ZOLOTOVA believes that if consideration is given to the density of the fruit pulp, the appropriate technique of sonication can be established for any fruit juice. She admits, nevertheless, that ultrasonic treatment promotes the decomposition of vitamin C in the fruit juice.

In this laboratory, fruit and vegetable juices were comminuted with various homogenizers, exposed to different effects. The suspensions thus obtained were subjected to dispersoid-analytical studies to establish the changes of particle size during comminution.

1. Materials and methods

1.1. Materials

Quince, plum, apricot and tomato juices were used in the comminution experiments.

Dispersoid-analytical studies are preconditioned by the knowledge of fibre density. The determination of the density of fibres swollen in an aqueous medium, as in this case, is problematic. Various authors, using different methods, obtained dissimilar results.

In the present experiments, fibre density was uniformly taken as 1.08 g/cm^3. This value was derived from picrometric measurement of the water-vapour absorption of fibres dehydrated by freeze-drying. As the steam-absorption process took several weeks, only few data were obtained.

SITNIKOV (1964) obtained different results, but within the same order. He found for tomato fibres a relative density of 1.05 g/cm^3 in an aqueous medium.

Apart from pointing out the uncertainity of density measurements, it should be noted that as long as the investigator confines himself to the establishment of relative correlations, no error is likely to be committed. Aiming to arrive at absolute conclusions, it should be always kept in mind that the

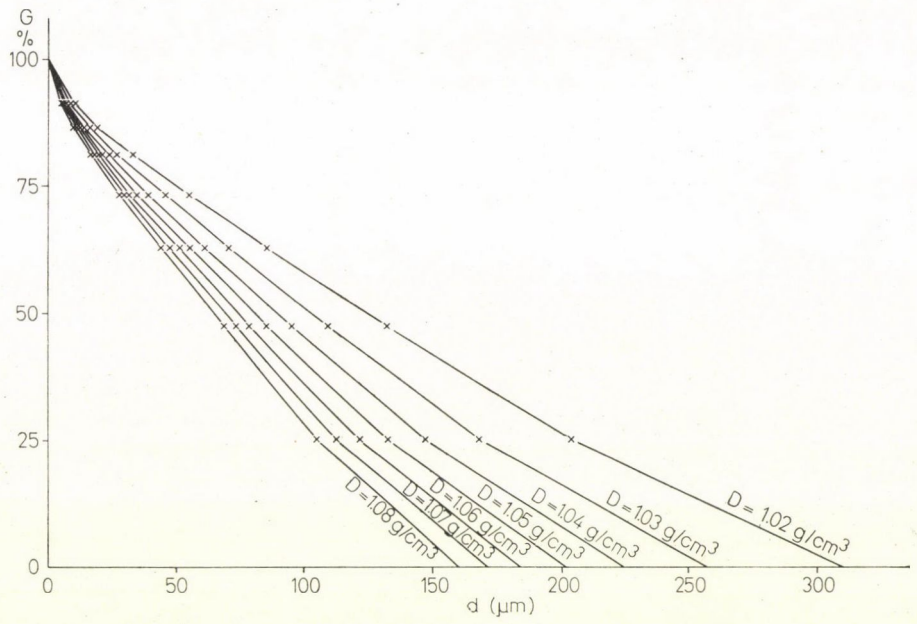

Fig. 1. Granulometric curves for quince fibre suspensions of various fibre densities

calculated particle diameters are functions of the density which, at the moment, cannot be precisely determined. As soon as the problem of density measurement can be resolved, these values, which now have to be treated with reservation, can easily be modified by an appropriate correction factor.

To illustrate this problem, granulometric curves for quince fibre suspensions of different fibre densities are presented in Fig. 1.

For the experiments, the particles of fruit and vegetable suspensions were dispersed in pure distilled water, to eliminate the interference of dissolved substances with the dispersoid-analytical measurements. The suspension was prepared as follows: the juice was centrifuged at 2800 r.p.m. for 25 minutes, the pure (particle-free) dispersoid medium was decanted, distilled water of roughly the same volume was added to the sediment and it was stirred and centrifuged again. This manipulation was twice repeated resulting in a pasty

substance to which 1% formaldehyde was added for preservation. With its fibre density determined, this material served as a stock suspension ready for use at any time.

1.2. Methods

1.2.1. Comminutors. Two devices, both based on ultrasonic effect were used for the comminution experiments. The experimental suspensions were prepared in the above way and were adjusted to the concentration required by the construction of the machine; they always consisted of fibres and distilled water only.

"*Minisonic*" *Ultrasonic Homogenizer.* The "Minisonic model 3" machine of the Ultrasonic Ltd. was used.

In this type of homogenizer the ultrasound is produced by a blade which vibrates at its natural frequency in a high-speed jet of the suspension to be dispersed, produced by a pump. The cavitation occurring in the vicinity of the blade releases instantly and locally high cavitation pressure, resulting in the dispersion of the suspended solid particles. The frequency of the ultrasonic vibrations is 18—22 Kc/sec. The capacity of the equipment is 1 litre/minute.

0.03% suspensions were comminuted throughout; they were jetted 1, 2, 4 or 8 times and the quince suspension even 16 times.

MSE Ultrasonic Disintegrator. The ultrasound is produced by a titanium probe connected by a transducer head with a signal generator of 18—20 Kc/sec frequency and placed in an acoustically insulated chamber. The output of the machine is 60 W (Measuring and Scientific Equipment Ltd; 1960).

Small cavitation bubbles form around the probe producing a powerful dispersing effect.

The specimens treated in the machine were 0.48% suspensions of 80 ml placed in a 150 ml beaker and treated for 1, 2, 4 or 8 minutes.

The suspensions of different concentrations as used in the two machines were necessitated by constructional differences.

1.2.2. Method of the dispersoid-analytical examinations. The distribution of particle sizes was established by the sedimentation technique elaborated in this department (URBÁNYI, 1968).

The measurements were performed at 20 ± 0.1 °C, using suspensions of 0.03% dry fibre content. The particle diameters selected as points of measurement were 160—105—68—44—28—17—10—5 μm.

1.3. Representation and evaluation of results

The granulometric data and the corresponding standard deviations were calculated from the dispersoid-analytical measurement.

The particle size distribution of the dispersoid systems was characterized numerically by Trask's parameters:

Q_1: *lower quartile*, the particle diameter below which there is 25% of the particles;

Q_3: *upper quartile*, the particle diameter below which there is 75% of the particles;

Md: median, the particle diameter dividing the particles in 50% to smaller and larger ones respetively. The change of the median (ΔMd) was calculated as per cent of the median obtained at the preceding lower degree of comminution and from this was derived the median for unit comminuting action (ΔMd_u), viz. one jetting/minute.

The results obtained at different degrees of comminution were analysed mathematically by Student's t test, to establish whether or not the granulometric data obtained with the increase of the degree of comminution differed significantly. This was intended to determine the degree up to which the operation of a given homogenizer is economic.

Again Student's t test was used to identify that degree of comminution at which the granulometric data differ significantly from those obtained in the original system. In these calculations, significance was established at a 95% probability level at least and the lowest degree of comminution causing a significant change at this level was designated as Z.

The results are shown in the attached Tables and Figures. The values for the diameters (d) always signify equivalent diameters.

2. Results

2.1. Examination of the comminution procedure

2.1.1. Examinations of fibre-suspensions homogenized with the Minisonic machine. Comminution of quince fibre suspension. Granulometric curves for the quince fibre suspensions, passed through the homogenizer 1, 2, 4, 8 and 16times, respectively, are shown in Fig. 2 and the results of mathematical-statistical analysis in Table 1.

The granulometric curves show that, as expected, with the increase of the number of treatments the size of the dispersed particles shifted toward the smaller dimensions. The comminuting effect tended to decrease with the increase of the degree of dispersity in the suspension; this can be explained by the greater comminution resistance of the smaller particles. This tendency is particularly well illustrated by the changes of the parameters (Fig. 3). A single jetting of the system reduced the median from the original 64.2 μm to 44.1 μm that is by 31.31%, the second treatment by 15.42%; the median

obtained after eight treatments changed only by 2.42% during additional eight treatments which makes a unit change (ΔMd_u) of only 0.28%.

Next the granulometric data obtained in the rising sequence of comminution treatments were examined for the significance of the differences between

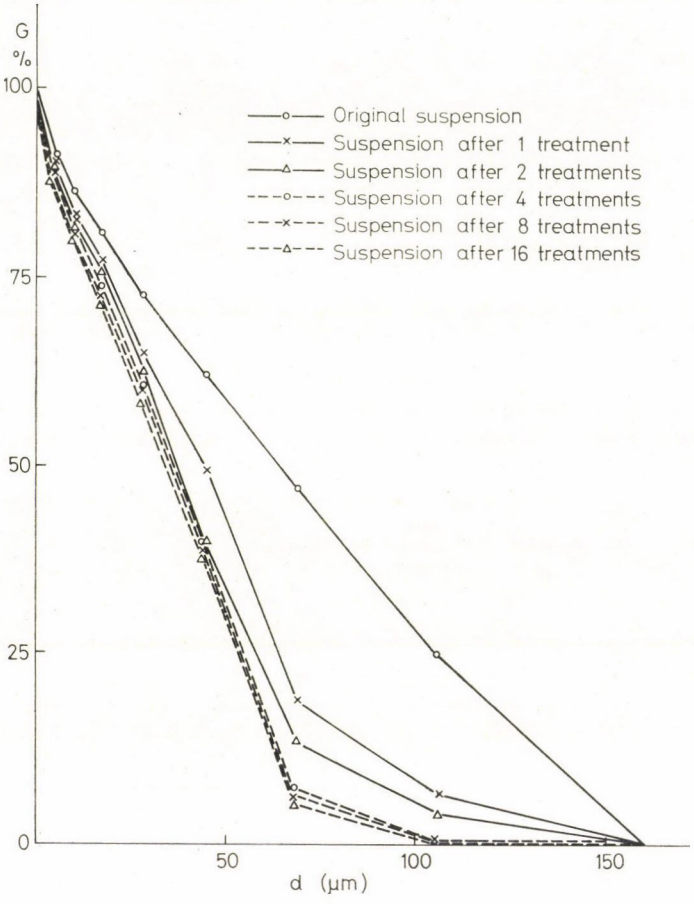

Fig. 2. Granulometric curves for quince fibre suspensions comminuted in the Minisonic ultrasonic disintegrator

them (Table 1). After the first treatment the values differed significantly from the original ones, except at the 5 μm level. On further exposures there was a gradual shift toward finer particle distributions, but the differences were significant only at a few points. Between the suspensions treated 8 or 16times there was no significant difference at any point.

On examining the comminuting effect which first produced granulometric data significantly differing from the original values it was found that two treatments on the Minisonic machine met this requirement. Of the seven points

Table 1

Statistical analysis of dispersoid-analytical data for quince fibre suspensions comminuted in the Minisonic apparatus

($P\%$ = level of significance; Z = number of treatments required to produce a particle size change differing from the initial size distribution at 95% probability level, at least)

d μm	Level of significance of the difference between the applied treatments ($P\%$)					Z
	Number of treatments					
	0—1	1—2	2—4	4—8	8—16	
160	—	—	—	—	—	—
105	>99.9	90.0	99.0	50.0	<50.0	1
68	>99.9	98.0	95.0	<50.0	50.0	1
44	>99.9	>99.9	0.0	75.0	<50.0	1
28	>99.9	90.0	75.0	90.0	50.0	1
17	>99.9	50.0	90.0	>99.9	50.0	1
10	>99.9	50.0	75.0	75.0	<50.0	1
5	<50.0	99.0	90.0	<50.0	50.0	2

of measurement, six differed significantly from the initial values already after the first treatment and the seventh (5 μm) after the second.

Comminution of plum fibre suspension. The data of the comminution of plum fibre suspension obtained in the Minisonic apparatus are shown in Fig. 4.

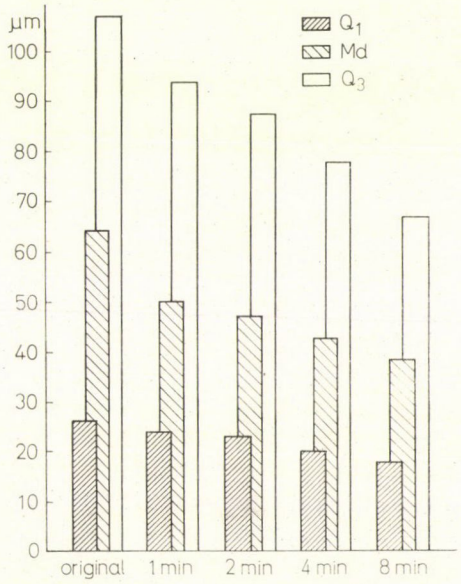

Fig. 3. Trask parameters of quince fibre suspensions comminuted in Minisonic apparatus

Here too, with increasing number of treatments the particle sizes shifted toward the smaller ones but the changes were of lesser extent than with the quince suspension.

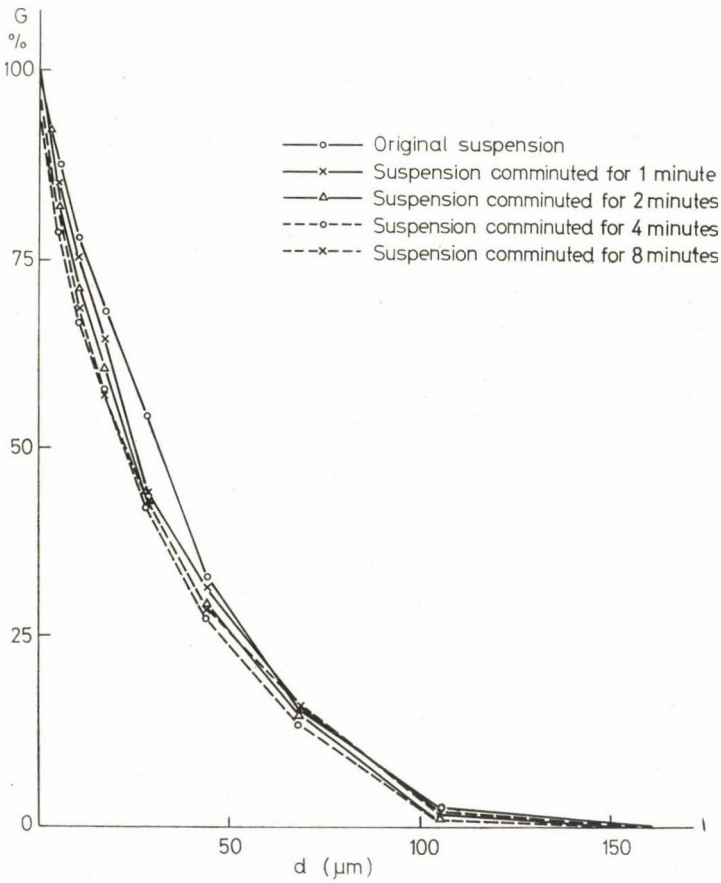

Fig. 4. Granulometric curves for plum fibre suspensions comminuted in Minisonic apparatus

As can be seen from the changes of the parameters (Fig. 5) the major part of the change occurred already during the first treatment when the median fell from 31.5 μm to 20 μm ($\Delta\, Md = 34.31\,\%$). The degree of comminution was much lower in the subsequent treatments the unit change being only 2.57 % in the last phase.

The mathematical evaluation of the results of the individual treatments (Table 2) revealed that the granulometric data of the specimens, passed over the Minisonic apparatus once, differed significantly for particles of 28 μm or smaller diameter. Between the first and second, the 2nd and 4th treatments,

respectively, the difference was significant only at one point, thus the comminuting effect is negligible. It is of interest that the differences between the 4th and 8th treatments were again significant within the particle size range below 28 μm.

Mathematical analysis performed to establish the degree of comminution producing significant difference from the original level at all points of measure-

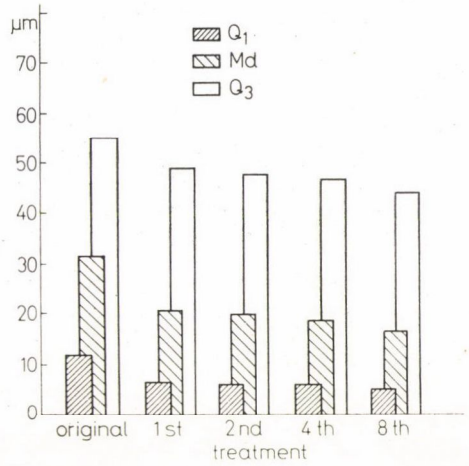

Fig. 5. Trask parameters for plum fibre suspensions comminuted in Minisonic apparatus

Table 2

Statistical analysis of dispersoid-analytical data for plum fibre suspensions comminuted in the Minisonic apparatus

(P% — level of significance; Z — number of treatments required to produce a particle size change differing from the initial at least at 95% probability level)

d μm	Level of significance of the difference between the applied treatments (P %)				Z
	Number of treatments				
	0—1	1—2	2—4	4—8	
160	—	—	—	—	—
105	75.0	<50.0	<50.0	<50.0	—
68	<50.0	<50.0	50.0	50.0	—
44	90.0	50.0	<50.0	50.0	8
28	>99.9	<50.0	<50.0	99.0	1
17	>99.9	98.0	75.0	95.0	1
10	>99.9	75.0	99.0	> 99.9	1
5	>99.9	50.0	75.0	99.8	1

ment has shown that even as much as 8 treatments failed to satisfy this criterion. The first treatment effected highly significant comminution in the particle size range below 28 μm and 8 treatments met this requirement at the 44 μm size level, but no such change occurred with the particles above that size (68 μm, 105 μm).

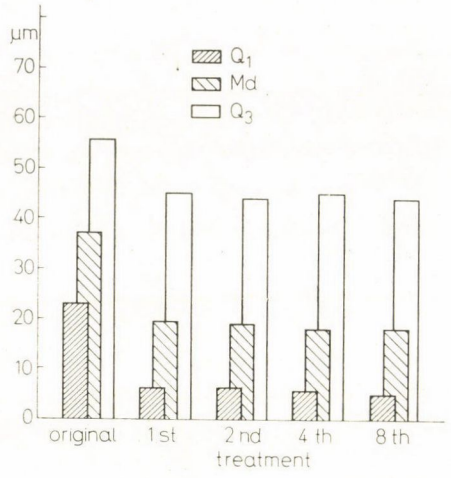

Fig. 6. Trask parameters for apricot fibre suspensions comminuted in Minisonic apparatus

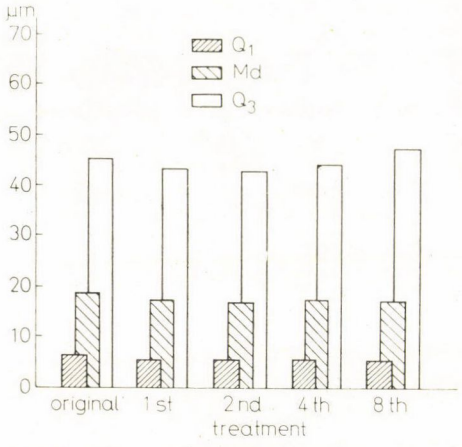

Fig. 7. Trask parameters for tomato fibre suspensions comminuted in Minisonic ultrasonic apparatus

Comminution of apricot fibre suspension. The degree of comminution was similar to that obtained with the plum fibre suspension (Fig. 6).

The changes of the median (ΔMd) at the successive degrees of comminution and the unit changes (ΔMd_u) derived from each are listed below in a rising sequence:

ΔMd:	47.73%	4.11%	3.75%	0.56%
ΔMd_u:	47.73%	4.11%	1.87%	0.14%

Also the mathematical-statistical analysis produced results similar to those obtained for the plum fibre suspension.

Comminution of tomato fibre suspension. With this suspension, the different degrees of homogenization produced little change in the particle sizes and no general trend of comminution was shown.

As shown by mathematical analysis (Table 3), a single treatment effected a significant change only at two points of measurement (105 μm, 17 μm).

The virtual increase of the particle sizes after the 4th and 8th treatments suggests agglomeration, but as this reverse trend of change was not significant at any point, it can not be regarded as proven.

None of the degrees of comminution tested could produce a suspension that differed significantly from the original particle size distribution at all points of measurement.

Table 3

Statistical analysis of dispersoid-analytical data for tomato fibre suspensions comminuted in the Minisonic apparatus

($P\%$ — level of significance; Z — number of treatments required to produce a particle size change differing from the initial at least at 95% probability level)

d μm	Level of significance of the difference between the applied treatments ($P\%$)				Z
	Number of treatments				
	0—1	1—2	2—4	4—8	
160	—	—	—	—	—
105	98.0	50.0	50.0	<50.0	1
68	75.0	75.0	50.0	<50.0	—
44	75.0	<50.0	50.0	50.0	—
28	75.0	<50.0	<50.0	<50.0	—
17	95.0	50.0	<50.0	<50.0	1
10	90.0	75.0	<50.0	50.0	2
5	90.0	<50.0	50.0	50.0	4

2.1.2. Examination of fibre suspensions comminuted with the MSE ultrasonic disintegrator. In this machine samples from all juice types were homogenized for 1, 2, 4 and 8 minutes and the suspensions thus obtained were compared to the original specimen and to one another.

Comminution of quince fibre suspension. Granulometric curves for the quince fibre suspensions, disintegrated for different periods are shown in Fig. 8 and the corresponding data of mathematical statistical analysis in Table 4.

The results clearly indicate the degree of comminution produced by the MSE ultrasonic disintegrator. Granulometrically, the 1-minute treatment produced a considerable change as compared to the original. The granulometric data of the suspensions treated for 1 and 2 minutes respectively, were almost identical and the subsequent exposures again resulted in a notable reduction of particle sizes.

The above tendencies are clearly apparent also from the changes of the parameters (Fig. 9); these decreased with the increase of the time of exposure, but their alteration was non-linear. The change taking place in unit comminution time tended to diminish with the increase of the degree of dispersity as indicated by the data shown below in the rising sequence of comminution times:

ΔMd_u: 21.66% 6.76% 4.48% 2.40%

The mathematical analysis of the results confirmed these conclusions.

Apparently, the MSE apparatus requires a relatively long exposure time to comminute quince suspensions to a satisfactory degree. Treatment for one or two minutes does not produce the desired result. There is reason to suppose

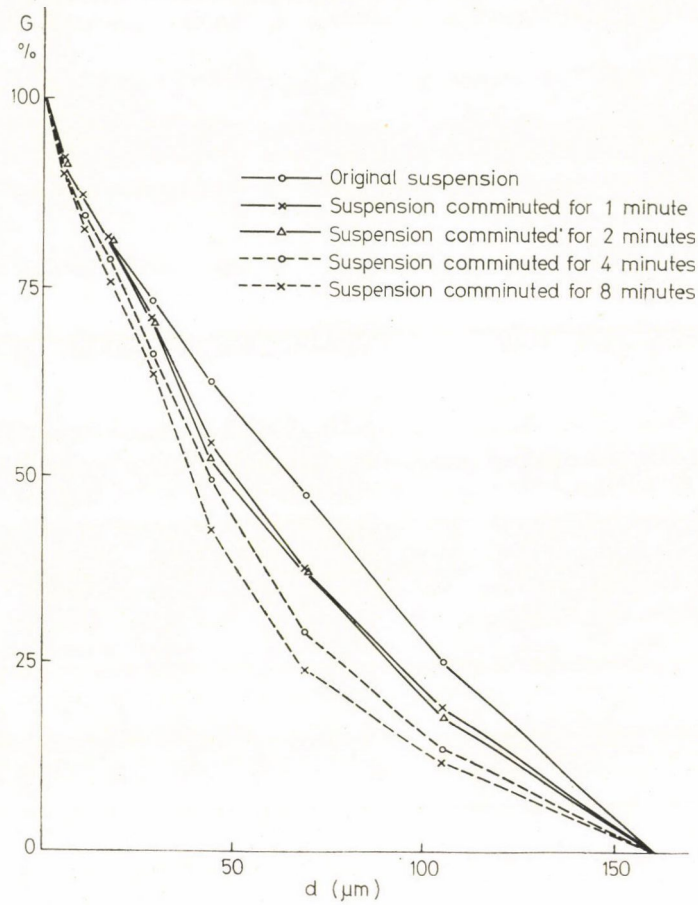

Fig. 8. Granulometric curves for quince fibre suspensions comminuted in MSE ultrasonic disintegrator

that even the longest exposure time applied in the experiment (8 minutes) failed to bring about the maximum degree of comminution obtainable with quince juice.

Comminution of plum fibre suspension. The results of the comminution of plum suspensions in a MSE apparatus are shown in Fig. 10.

The results were similar to those obtained with the quince suspension, but the longest exposure (8 min.) effected a reduction of the degree of dispersion. This suggested the predominance of agglomeration.

Table 4

Statistical analysis of dispersoid-analytical data for quince fibre suspensions comminuted in the MSE ultrasonic disintegrator

(P% — level of significance; Z — number of treatments required to produce a particle size change differing from the initial at least at 95% probability level)

d μm	Level of significance of the difference between the applied treatments (P %)				Z
	Comminution times (min.)				
	0—1	1—2	2—4	4—8	
160	—	—	—	—	—
105	>99.9	50.0	98.0	75.0	1
68	>99.9	99.0	95.0	99.0	1
44	>99.9	99.0	>99.9	>99.9	1
28	99.0	<50.0	98.0	90.0	1
17	<50.0	<50.0	98.0	98.0	4
10	50.0	<50.0	99.8	99.0	4
5	75.0	<50.0	90.0	75.0	—

The statistical analysis of the granulometric data (Table 5) showed that the suspension treated for one minute differed significantly from the original one for particle sizes of 28 μm and below. Comparison of the results of exposures

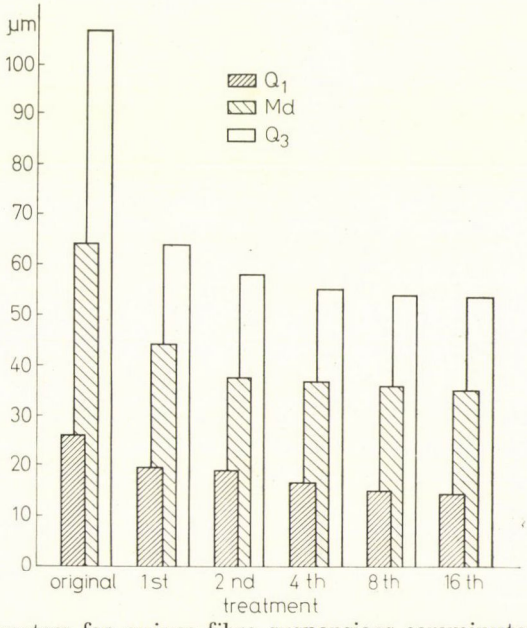

Fig. 9. Trask parameters for quince fibre suspensions comminuted in MSE ultrasonic disintegrator

for 1—2 and 2—4 minutes, respectively, revealed significant differences at most points of measurement, but not at all of them.

Granulometrically, the suspensions treated for 4 and 8 minutes differed significantly at only three points of measurement, thus there is no proof of agglomeration.

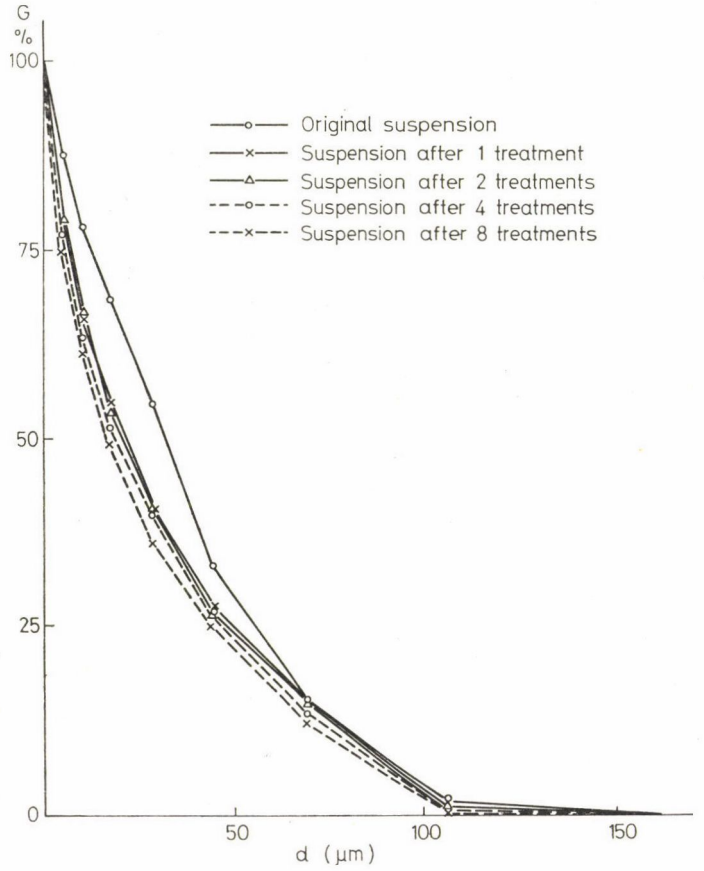

Fig. 10. Granulometric curves for plum fibre suspensions comminuted in MSE ultrasonic disintegrator

As shown by the Z-values, the applied treatments failed to produce a suspension showing a significant diminution of particle size at each point of measurement. The data of the 8-minute exposure suggest that no such result is likely to be produced by increasing the time of disintegration beyond that upper limit.

Comminution of apricot fibre suspension. The results were similar to those obtained for the plum fibre suspensions. Exposure for 8 minutes resulted in the increase of the particle size also here, as shown by the parameters (Fig. 11).

Table 5

Statistical analysis of dispersoid-analytical data for plum fibre suspensions comminuted in the MSE ultrasonic disintegrator

(P% — level of significance; Z — number of treatments required to produce a particle size change differing from the initial at least at 95% probability level)

d μm	Level of significance of the difference between the applied treatments (P %)				Z
	Comminution times (min.)				
	0—1	1—2	2—4	4—8	
160	—	—	—	—	—
105	<50.0	75.0	<50.0	98.0	—
68	<50.0	75.0	75.0	95.0	—
44	50.0	95.0	95.0	75.0	4
28	98.0	90.0	95.0	50.0	1
17	95.0	99.0	90.0	<50.0	1
10	98.0	99.0	99.0	90.0	1
5	99.8	>99.9	99.0	98.0	1

The mathematical analysis of the granulometric data confirmed the above observations. Between the exposures for 4 and 8 minutes, a significant increase of particle size occurred only at three points of measurement, thus there is again no firm proof of agglomeration.

Comminution of tomato fibre suspension. According to the experimental results, the applied treatment produced no notable comminution (Fig. 12). Exposure for 1 minute resulted in a slight decrease of all the three parameters,

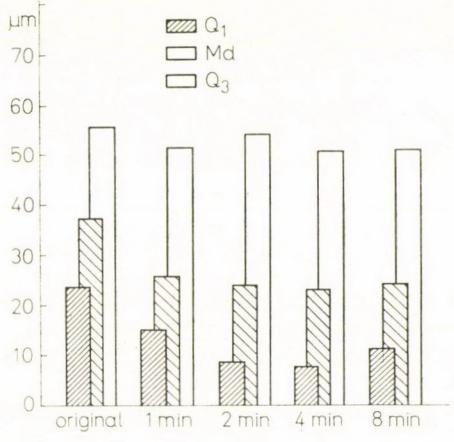

Fig. 11. Trask parameters for apricot fibre suspensions comminuted in MSE ultrasonic disintegrator

but the prolongation of the treatment brought about a very slight increase instead of decrease of the particle size.

The change effected by treatment for one minute was significant only at two points of measurement and the results of disintegration for longer times did not differ significantly at any point.

Comparison with the original suspension of the systems obtained by disintegration for various times showed that the increase of the time of

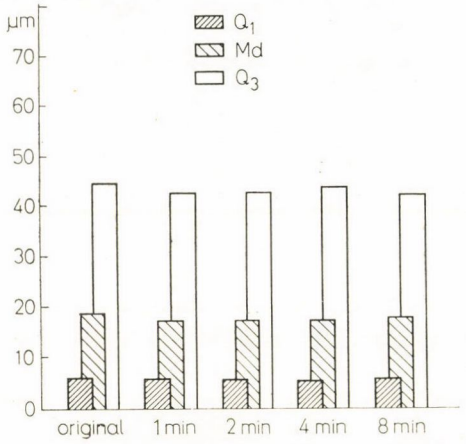

Fig. 12. Trask parameters for tomato fibre suspensions comminuted in MSE ultrasonic disintegrator

comminution failed to bring about a significant reduction of particle size over the entire range observed.

The conclusion remains to be drawn, therefore, that the MSE apparatus is unsuitable for further disintegration of systems of already high degree of dispersity.

3. Discussion

3.1. Conclusions drawn from the experiments performed with two different types of disintegrators

3.1.1. Comminution experiments in the Minisonic apparatus. Experiments in this apparatus with four different kinds of samples have shown that ultrasonic treatment effected a shift of the granulometric data toward a finer particle size distribution and that the substantial part of comminution took place always during the first treatment decreasing gradually with the increase of the degree of dispersity of the system. This explains the significant change of the granulometric data at most points of measurement during the first treat-

ment of the originally coarsely distributed quince, plum and apricot fibre suspensions, as contrasted to the slight, statistically not significant change of the particle size of the tomato fibre suspension which had *ab ovo* been highly dispersed. The dispersity of the original tomato fibre suspension was close to that of the other three specimens in maximally comminuted state; the parameters were almost identical, as shown below:

	Q_1	*Md*	Q_3
Plum, after 8 treatments	5.0	16.6	44.2 μm
Apricot, after 8 treatments	5.0	17.9	43.9 μm
Tomato, original	5.9	18.3	44.8 μm
Tomato, treated twice (minimal particle sizes)	5.0	16.5	42.7 μm

This suggests that suspensions of the above particle size distribution become "resistant" to further disintegration by the Minisonic apparatus, responding by agglomeration of the particles rather than by a further reduction of their sizes.

3.1.2. Comminution experiments with the MSE ultrasonic apparatus. Like in the Minisonic apparatus, the granulometric data of the examined suspensions varied with the degree of comminution. Here, too, the greater part of comminution took place during the first minute of the first treatment, the changes of the medians in quince, plum and apricot fibre suspensions being 21.66, 16.83 and 31.64%, respectively, and never exceeding 10% during the subsequent exposures. As the degree of dispersity of the tomato fibre suspension was initially high, the change of its median was only 6.56% in the first minute.

The above data suggest that the smaller the initial particle size, the greater the resistance to disintegration, that is, the more the system approximates the state of agglomerational equilibrium, the less it will yield to further comminution.

The parameters of the suspensions showing the highest degrees of comminution were the following:

	Q_1	*Md*	Q_3
Quince, disintegrated for 8 minutes	17.9	38.6	67.4 μm
Plum, „ „ 4 „	6.6	22.5	48.3 μm
Apricot, „ „ 2 „	7.5	22.8	50.5 μm
Tomato, „ „ 2 „	5.3	17.0	44.7 μm

Under the given experimental conditions no maximal comminution could be obtained with the quince fibre suspension; the granulometric data for the 4-minute and 8-minute exposures also differed significantly.

3.2. Comparison of the disintegrators tested

To characterize the maximal comminuting effect, C_{25}, C_{50}, and C_{75} values were determined for the two apparatuses, which are defined as follows:

C_{25} = quotient of the lower quartiles of the untreated and maximally comminuted systems;

C_{50} = quotient of the medians for the untreated and maximally comminuted systems;

C_{75} = quotient of the upper quartiles for the untreated and maximally comminuted systems.

"C" is understood as comminution; $C = 1$ means that no comminution took place that is, the agglomeration equilibrium has become established.

The "C" values calculated for the four suspensions examined after treatment in the two kinds of apparatus are shown in Table 6.

Table 6

C (comminution) values established for the examined fibre suspensions in the two ultrasonic comminutors tested

Fibre suspension	Comminutor					
	Minisonic			MSE ultrasonic		
	C_{25}	C_{50}	C_{75}	C_{25}	C_{50}	C_{75}
Quince	1.8055	1.8343	1.9888	1.4525	1.6632	1.5846
Plum	2.4400	1.8976	1.2421	1.8484	1.4000	1.1366
Apricot	4.6800	2.0838	1.2665	3.1200	1.6360	1.1010
Tomato	1.2040	1.1091	1.0492	1.1132	1.0765	1.0467

As can be seen from the Table, the Minisonic apparatus had the greater comminuting effect of the two; the C-values for the Minisonic were always higher than those for the MSE ultrasonic disintegrator.

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IMPROVEMENT OF POLYGALACTURONASE PRODUCTION OF ASPERGILLUS STRAINS BY UV-IRRADIATION

K. ZETELAKI-HORVÁTH and I. DOBRA-SERES

(Received September 10, 1970)

Spores of *Aspergillus awamori* and *Aspergillus niger* were irradiated with UV-lamps to enhance their polygalacturonase (SPA_{75}^{Obi}) production and apple juice clarifying (SPA_{75}^{A}) activity. Out of 244 *Aspergillus awamori* morphological mutants isolated after irradiation, 34 proved to be better producing mutants for polygalacturonase. More than twofold increase in polygalacturonase production was found in three of these cases as compared with the activity of the original strain (SPA_{75}^{Obi} = 2000 l/h/l). Strains revealing the best apple juice clarifying activity in sucrose containing culture media, were irradiated repeatedly.

The best apple juice clarifying activity obtained in pectin containing (induction) media was more than the treble of the activity of non-irradiated strains (SPA_{75}^{A} = 100 l/h/l).

The enzyme production (SPA_{75}^{Obi} or SPA_{75}^{A}) of the variants showed improvement after irradiation of the *Aspergillus niger* strain, however, the levels obtained were essentially lower than those of the *Aspergillus awamori* strains.

A great number of microorganisms show sensitivity to ultraviolet radiations (ALFÖLDY et al., 1969; KONICKOVÁ-RADOCHOVÁ & MÁLEK, 1969; HORVÁTH, 1970), and to ionizing radiations (GUDA et al., 1962; SASAKI et al., 1969).

260 nm wavelength proved to be the most effective for the majority of fungi (POMPER, 1965). The UV-lamps can be successfully applied for disinfection because of the strong bactericidal effect of the ultraviolet rays (STIFF, 1969). Different mutations can be provoked, if UV rays are applied below the lethal dose. The occurrence of the mutations can be ascribed to strong UV-absorption of the nucleic acids, i.e. to the photochemical reactions it causes.

The UV-radiation exerts its effect mainly through the deoxyribonucleic acid of the cell nucleus. It has been found (DEERING, 1962) that the pyrimidine bases of DNA (thymine and cytosine) are more sensitive to UV-light and change more rapidly to its effect than the purine bases (adenine and guanine).

The recognition of the mutagenic effect of UV radiation initiated series of genetic works (KELNER, 1948; SERMONTI et al., 1956; BRAENDLE et al., 1957, 1959; JÁRAI, 1961a, b; BUXTON, 1956; HASTIE, 1962) with the purpose of altering different characteristics of the microorganisms (BUXTON, 1956; LEWIN, 1960; KONICKOVÁ-RADOCHOVÁ & MÁLEK, 1969).

The mutagenic effect of the radiations is frequently used in the biosynthesis industries to produce plus variants (ALIKHANJAN et al., 1970).

Aspergillus awamori and *Aspergillus niger* strains have been irradiated by germicide lamps in our experiments to enhance their polygalacturonase enzyme production and the biosynthesis of the enzyme components active in clarifying apple juice.

1. Materials and methods

1.1. Microorganisms applied

P-1925 *Aspergillus awamori* and *Aspergillus niger* No. 34 strains have been used in our experiments.

1.2. UV-irradiation

The irradiation of the strains mentioned above was performed with a germicidal lamp ("Labor" product). The spectrum of the mercury-vapour lamp (type: PRK-2) consisted of rays between 240 and 580 nm, 90% of which was of 253 nm.

The irradiation was carried out in Petri dishes of 7.2 cm diameter with 4 ml of a spore suspension of 10^8/ml from a distance of 15 cm. After 20 to 50 minutes of exposure time inoculations were made on malt agar in Petri dishes. Morphological mutants were isolated from the colonies developed after 4 days incubation at 28 °C.

1.3. Culturing of the isolated mutants under submerged conditions

The polygalacturonase production of the morphological mutants was examined in shake-cultures, after incubation for 48 hours at 28 °C. A shaking-machine operating with 330 r.p.m. (length of stroke: 2.4 cm) provided 17—19 mMol O_2/l/hour of oxygen supply for aerating the cultures.

1.4. Culture media applied

1.4.1. Malt agar. Malt agar was used for the maintenance of the strains and to disperse the spores irradiated. (2% agar-agar in a malt solution adjusted to 5% refractive index.)

1.4.2. Fermentation media. The composition of the media for the shake-cultures was the following:

Medium with sucrose: sucrose 7%, $(NH_4)_2SO_4$ 2%, corn-steep liquor 1%, KH_2PO_4 0.2%, pH 3.5.

Medium with pectin: pectin 2%, $(NH_4)_2SO_4$ 2%, corn-steep liquor 1%, KH_2PO_4 0.2%, pH 4.5.

1.5. Methods for the determination of enzyme activity

1.5.1. Determination of polygalacturonase enzyme activity. The polygalacturonase activity was measured by viscometry and expressed in terms of Specific Pectolytic Activity (SPA_{75}).

The value of SPA_{75} represented the quantity of substrate decomposed by 75% of its original specific viscosity by 1 litre of culture filtrate during 1 hour incubation at 50 °C (VAS, 1953).

1.5.2. Determination of apple juice clarifying enzyme activity. The apple juice clarifying activity of the cultures was measured by the decrease of the initial specific viscosity of the natural apple juice. The original specific viscosity of the juice of Jonathan apples used was 1.55. The apple juice clarifying activity (SPA_{75}^{A}) was characterized by the quantity of apple juice, the pectin content of which was reduced to 75% by 1 l culture filtrate during 1 hour incubation at 50 °C.

1.6. Detection of galacturonides by paper chromatography

To study the breakdown products of polygalacturonic acid decomposed by the enzyme, 1.0 ml of enzyme solution was added to 5 ml of 1% polygalacturonic acid, dissolved in 0.05 M acetate buffer (pH 4.2). The mixture was incubated for 5 hours at 30 °C, subsequently 30 µl was placed on Whatman paper No. 1 (size: 33 ×52 cm). The solvent used for ascending chromatography was butanol–acetic acid–water mixture (5 : 2 : 3) and the solution for developing was 0.5% bromophenol blue in 96% ethanol.

2. Results

The spore suspension (10^8 cells/ml) of *Aspergillus awamori* and *Aspergillus niger* strains had been irradiated for a definite time and subsequently inoculated on malt agar plates in Petri dishes. The number of survivors was determined after 6 days incubation. Fig. 1 shows the data of destruction of the strains mentioned above, caused by UV-irradiation. The logarithm of the number of survivors is indicated on the ordinate and the duration of irradiation on the abscis. Each data of the curves represents the average value of the number of colonies developed on the agar plates in 12 Petri dishes.

Fig. 1 shows that the *Aspergillus awamori* strain was more sensitive to UV-irradiation than *Aspergillus niger*.

The 20- and 30-minute exposition times proved to have the most favourable mutagenic effect. From the colonies developed after 96 hours incubation, mainly colonies with smooth surface and darker colour than that of the original and covered by spores of greyish-black colour were isolated. Fig. 2 demon-

strates the surface growth of the non-irradiated *Aspergillus awamori* strain and of one of the UV-variants, on agar-plate, after 10 days incubation at 28 °C.

The growth of the non-irradiated strain in the left Petri dish is slow, its spore formation weak, the colour of the spores is pale brownish-grey.

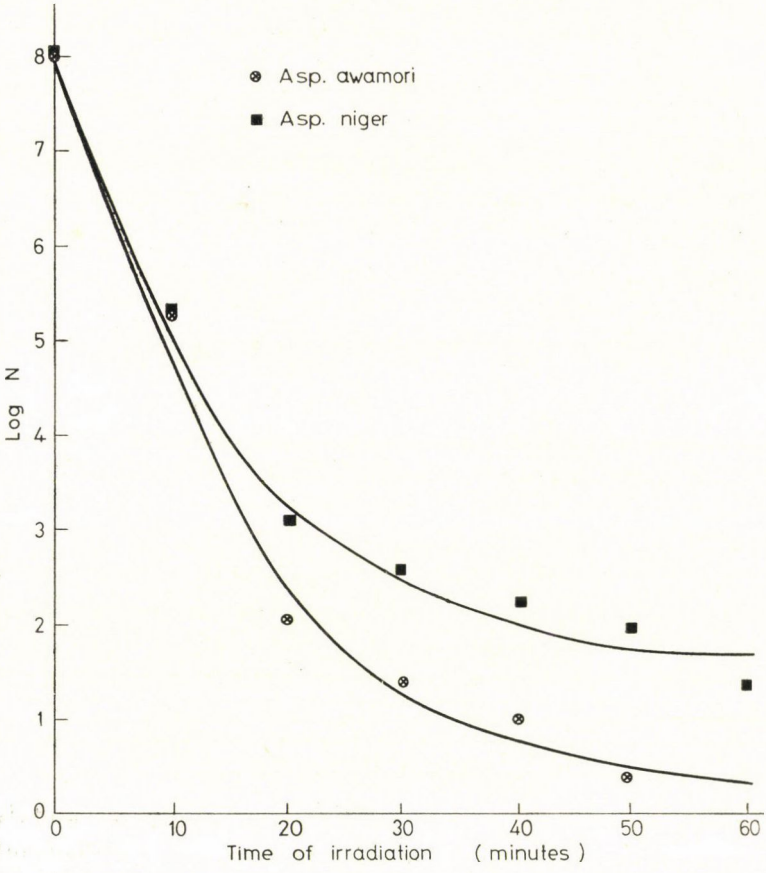

Fig. 1. Data on cell destruction caused by UV-irradiation, with *Aspergillus awamori* and *Aspergillus niger* strains, as a function of irradiation time

The growth of the UV-variant on the right side of Fig. 2 is more rapid, the spore formation stronger and the colour of the spores dark greyish-black.

The polygalacturonase production, the apple juice clarifying activity and the development of the isolated variants was examined in shake cultures after 48-hour incubation at 28 °C.

The activity values of the shake cultures of *Aspergillus awamori* morphological mutants, as measured on Obipektin substrate, are demonstrated in Fig. 3 in relation to the duration of irradiation. The horizontal line in the Figure indicates the level of polygalacturonase activity of the original strain.

Fig. 2. Colonies of *Aspergillus awamori* strain P-1925 and of its variant No. 282, produced by UV-irradiation. The cultivation was performed on agar plates, with a 10-day incubation at 28 °C

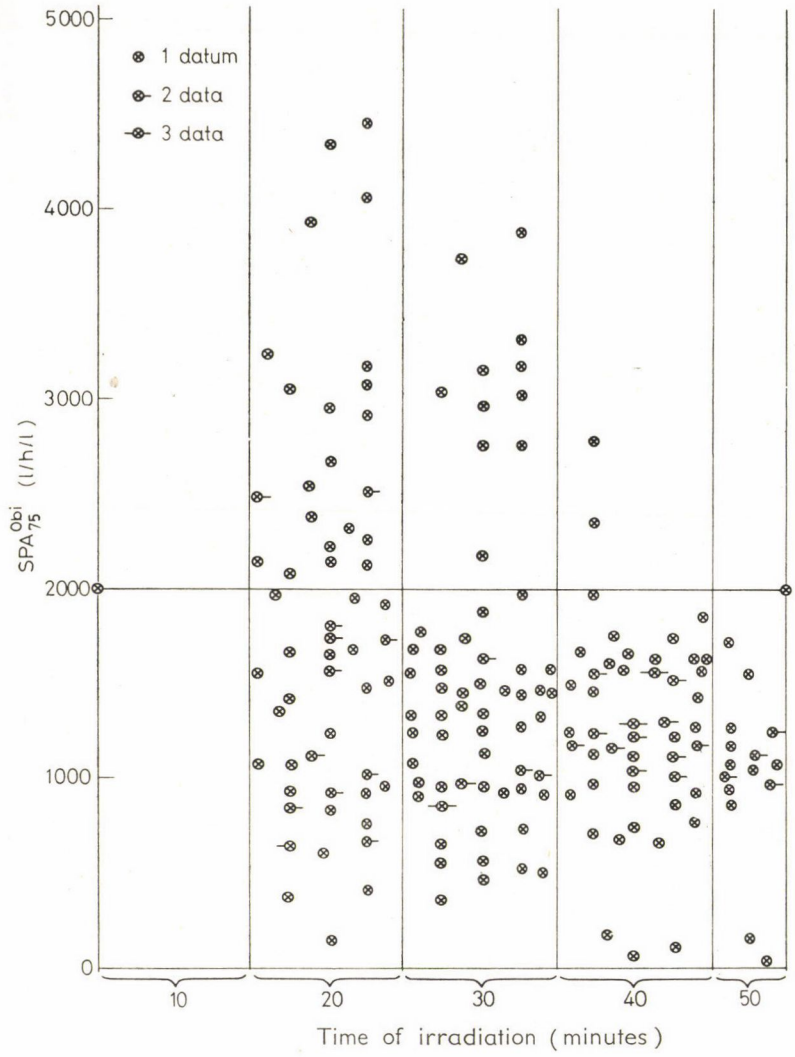

Fig. 3. Development of polygalacturonase concentration (SPA_{75}^{Obi}) of *Aspergillus awamori* morphological mutants isolated after UV-irradiation for different periods. Cultivation was performed in sucrose containing media, at 28 °C, by shaking the cultures for 48 hours. The horizontal line in the Figure indicates the activity of the original strain

Evaluating the data of our experiments it can be stated, that out of the 244 isolated *Aspergillus awamori* morphological mutants 34 revealed an improved ability to produce polygalacturonase. The polygalacturonase concentration of the culture fluid of 15 strains out of the 34 variants exceeded the value of $SPA_{75}^{Obi} = 3000$ l/h/l, 4 of them showed strikingly good capacity to produce polygalacturonase at a 3900—4500 l/h/l concentration level.

Fig. 3 shows, furthermore, that the best polygalacturonase producing variants are obtained by irradiating the spores for 20 minutes.

Out of the 244 *Aspergillus awamori* UV-variants, the distribution of the activity values of variants showing SPA_{75}^{A} levels higher than 10 l/h/l is presented in Fig. 4 in relation to the duration of irradiation. The horizontal line indicates the apple juice clarifying activity of the original strain.

It can be observed in Fig. 4 that the variants with the highest apple juice clarifying activity were obtained by irradiating the spores for 20 (3 variants), 40 (3 variants) and 30 (2 variants) minutes, respectively.

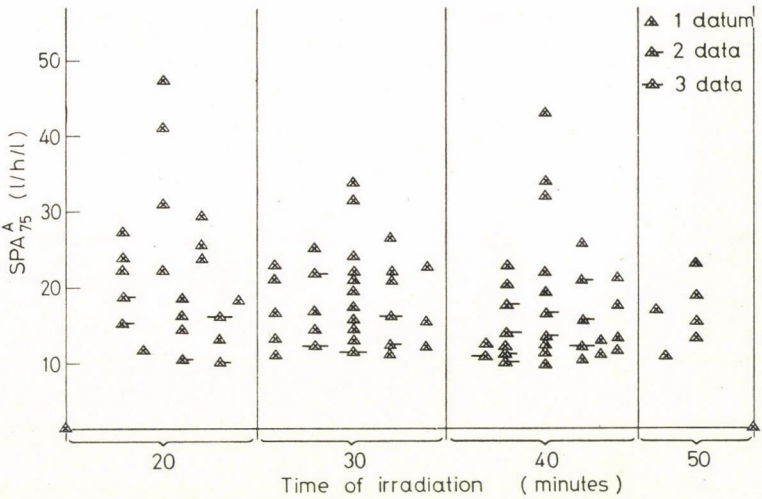

Fig. 4. Development of apple juice clarifying activity of (SPA_{75}^{A}) of *Aspergillus awamori* morphological mutants irradiated for different periods. The cultivation was performed in sucrose containing media, at 28 °C by shaking the cultures for 48 hours. Only data of variants showing SPA_{75}^{A} 10 l/h/l are demonstrated in the Figure. The horizontal line indicates the activity of the initial strain

Fig. 5 summarizes the development of polygalacturonase activity and apple juice clarifying activity (as measured on Obipektin substrate) of 130 *Aspergillus niger* morphological mutants isolated after ultraviolet irradiation. The levels of the polygalacturonase and the apple juice clarifying activity of the initial strain marked No. 34 are denoted with horizontal lines.

As it appears from this Figure, we succeeded only in doubling the polygalacturonase production in the UV variants of the low activity strain. The more active polygalacturonase producing variants were obtained mostly from the spores irradiated for 30 or 40 minutes. No variant with significant apple juice clarifying activity could be observed.

The polygalacturonase production and apple juice clarifying activity of the original and of some morphological mutants of *Aspergillus awamori* isolated after irradiation were studied in shake culture after 72 hours using

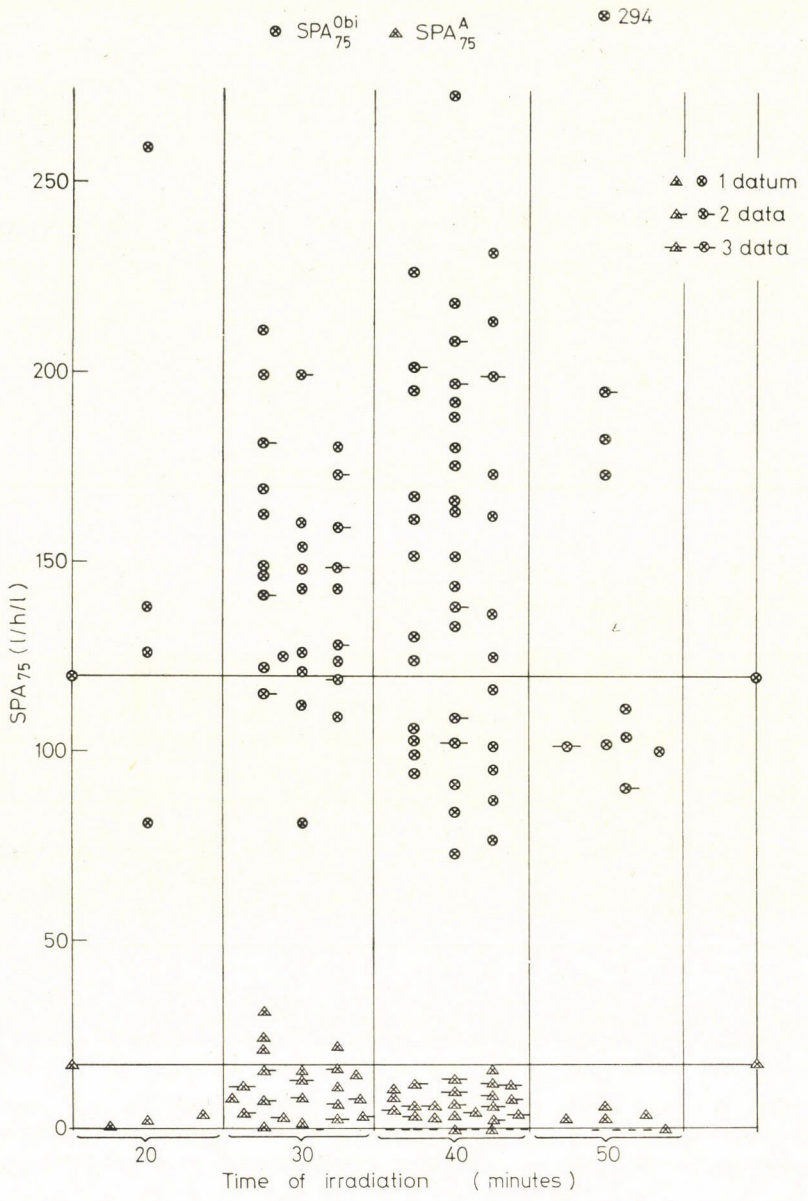

Fig. 5. Development of polygalacturonase (SPA_{75}^{Obi}) and apple juice clarifying (SPA_{75}^{A}) activities of *Aspergillus niger* morphological mutants irradiated for different periods. The cultivation was performed in sucrose containing media, at 28 °C, by shaking the cultures for 48 hours. The horizontal lines indicate the polygalacturonase and apple juice clarifying levels of the initial strain

pectin containing induction media. The polygalacturonase production (SPA_{75}^{Obi}) of the original strain was 181 l/h/l, its apple juice clarifying activity (SPA_{75}^{A}) 100 l/h/l on pectin containing media. The highest polygalacturonase activity of the isolated UV variants was 220 l/h/l and their clarifying activity 150 l/h/l as observed in pectin containing media. No significant differences could be found in the polygalacturonase production of the original strain and of the variants isolated after the first irradiation. Not more than 50% improvement could be obtained in the apple juice clarifying activity of irradiated strains.

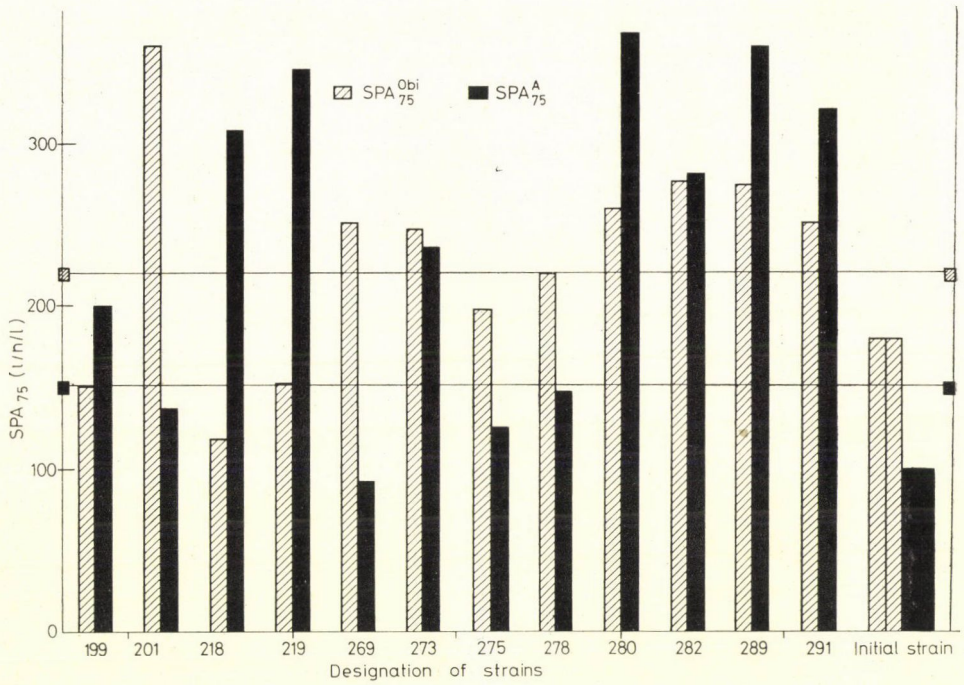

Fig. 6. Development of polygalacturonase (SPA_{75}^{Obi}) and apple juice clarifying (SPA_{75}^{A}) activities of the initial strain and that of the twice irradiated *Aspergillus awamori* UV-variants. The cultivation was performed in pectin containing media, shaking the culture for 72 hours at 28 °C. The horizontal lines indicate the maximum activity levels obtained after first irradiation

Seeing that the clarifying activity of the *Aspergillus awamori* variants improved as compared to the original strain (50% in some cases), the isolated variants were irradiated repeatedly. Twenty minutes of irradiation was applied with each variant. The production of the original and twice irradiated strains was compared in 72-hour shake cultures using pectin containing culture media. Fig. 5 shows the polygalacturonase and apple juice clarifying activity of the original and twice irradiated *Aspergillus awamori* strains. The horizontal lines

Table 1

Development of polygalacturonase production and apple juice clarifying activity of twice irradiated Aspergillus awamori strains, in 72-hour shake cultures, using pectin containing culture media

Designation of strains	SPA^{Obi}_{75}		SPA^{A}_{75}		Rate of polygalacturonic acid decomposition				
	l/h/l	Rate of activity as compared to the original strain	l/h/l	Rate of activity as compared to the original strain	mono-	di-	tri-	tetra-	penta-
					galacturonic acid				
Initial strain P 1925	181	—	100	—		++	++	++	+
199	151	0.83	200	2.00	+++	++	++		
201	360	1.99	137	1.37	++	++	++	+	
218	119	0.66	308	3.08	+++	+++	+	+	+
219	153	0.84	345	3.45	+++	+	+	+	+
269	251	1.39	92	0.92	+	+++	++		
273	247	1.36	236	2.36	++	+	+		
275	198	1.09	125	1.25	+++	++	++		
278	221	1.22	144	1.44	+++	++	++	+	
280	260	1.44	375	3.75	+++	+	+		
282	277	1.53	281	2.81	+++	++	+	+	
289	275	1.52	360	3.60	+++	++	+		
291	252	1.39	325	3.25	+++	+	+	+	

Note: Initial strain denotes non-irradiated strain.

in the Figure indicate the highest levels of polygalacturonase and apple juice clarifying activity attained after the first irradiation.

Data on enzyme activity and polygalacturonic acid decomposition of 72-hour *Aspergillus awamori* culture filtrates are summarized in Table 1 and Fig. 6.

It appears from Table 1 that only one and a half times greater polygalacturonase activity — as measured with Obipektin substrate — and more than three and a half times greater apple juice clarifying activity could be obtained than with the original strain. The rate of polygalacturonic acid decomposition was also highly increased with the twice irradiated strains. While the culture filtrate of the original strain decomposed the polygalacturonic acid only to digalacturonic acid, the majority of the polygalacturonic acid, incubated with the culture filtrate of twice irradiated strains was decomposed to monoga-

lacturonic acid. The other galacturonic acid oligomers gave essentially smaller spots in chromatograms.

The development of polygalacturonase production and apple juice clarifying activity of the twice irradiated *Aspergillus niger* variants, as studied in 72-hour shake cultures using pectin containing media, are presented in Fig. 7 and Table 2. The horizontal lines in the Figure denote the maximum activity levels obtained after the first irradiation.

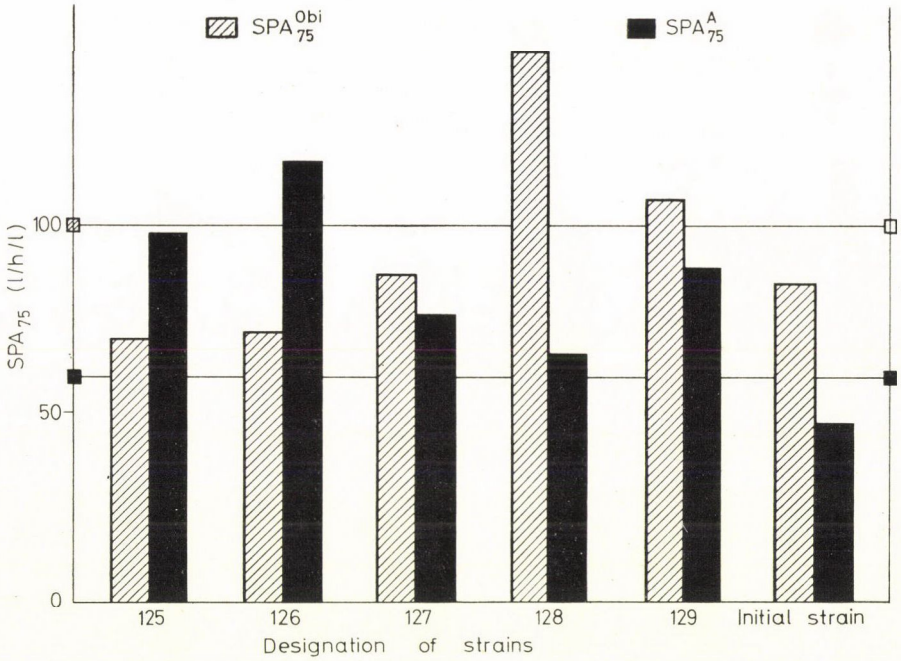

Fig. 7. Development of polygalacturonase (SPA_{75}^{Obi}) and apple juice clarifying (SPA_{75}^{A}) activities of the initial strain and that of the twice irradiated *Aspergillus niger* UV-variants. The cultivation was performed in pectin containing media, at 28 °C, shaking the cultures for 72 hours. The horizontal lines indicate the maximum activity levels obtained after first irradiation

According to the data of the Table, the greatest activity of the twice irradiated strains — as measured on Obipektin substrate — was 1.7 times greater, and their apple juice clarifying activity was 2.4 times greater than the activity of the original strain. The culture filtrate of the twice irradiated strains hydrolysed the polygalacturonic acid more completely, and decomposed greater part of it to monogalacturonic acid than with the original strain.

Table 2

Development of polygalacturonase production and apple juice clarifying activity of twice irradiated Aspergillus niger strains, in 72-hour shake cultures, using pectin containing culture media

Designation of strains	SPA^{Obi}_{75}		SPA^{A}_{75}		Rate of polygalacturonic acid decomposition				
	l/h/l	Rate of activity as compared to the original strain	l/h/l	Rate of activity as compared to the original strain	mono-	di-	tri-	tetra-	penta-
					galacturonic acid				
Initial strain 34	85		48		+	++	++	++	+
125	70	0.82	98	2.04	++	+	+	+	+
126	72	0.85	117	2.44	++	+	+	+	+
127	87	1.02	76	1.58	++	++	++	++	
128	146	1.72	66	1.37	++	++	++	++	
129	107	1.26	89	1 85	++	+++			

Rate of polygalacturonic acid decomposition

Note: Initial strain denotes non-irradiated strain.

3. Disscussion

The *Aspergillus awamori* strain, decomposing the prepared pectin with good effect, proved to be more sensitive to UV irradiation than the *Aspergillus niger* strain (Fig. 1). Fifteen strains out of the 34 variants isolated after irradiation, showed 50 %, and 3 strains higher than 100 % polygalacturonase yield increase, which result can be regarded as significant, considering the great activity of the original strain.

The apple juice clarifying enzyme activity of the irradiated strains showed likewise significant improvement, the activity of 8 strains showed 10fold increase compared to the original strain, after being cultured on sucrose containing media. Some of the strains revealing the best apple juice clarifying activity, were irradiated repeatedly.

After the second irradiation, we succeeded in isolating variants, the clarifying activity of which was more than three and a half times greater than that of the non-irradiated strain, this activity level was found to be more than the double of that measured after the first irradiation (Fig. 6).

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INVESTIGATIONS INTO THE POSSIBILITIES OF ENZYMATIC HYDROLYSIS OF CELLULOSE CONTAINING WASTES

PART I — THE EFFECT OF CELLULOLYTIC ENZYME PREPARATIONS ON ACID PRETREATED POPLAR WASTES

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Preliminary experiments were carried out to produce liquors containing reducing sugar by means of enzymatic hydrolysis of acid pretreated poplar sawdust.

1. Two different methods of sawdust pretreatment were applied: *a)* the sawdust was digested in the form of an aqueous suspension at 12 kp/cm² CO_2 gauge pressure and a temperature of 130 °C for two hours, or *b)* 1 part by weight of air dry sawdust was mixed with approximately 1.7 parts by weight (= 1 part by volume) of 75 w% sulphuric acid and after dilution with different amounts of water the mixture was heated on a 100 °C water-bath for 15 to 60 minutes. (The latter pretreatment was performed at the Department for Chemical Engineering of the Eötvös Loránd University of Sciences, Budapest).

2. The pretreated suspension corresponding to a sawdust concentration of 5 g per 100 ml of reducing sugar expressed in glucose, or 5 to 8% related to the dry matter content of the sawdust.

3. The liquid and slurry phases of the sawdust suspensions obtained after predigestion were separately hydrolyzed with 8 types of commercial cellulase preparations (Table 1). The enzyme preparations were added in three concentrations: 1, 3 and 10% were added related to dry sawdust, corresponding in the liquid phase to 0.06, 0.18 and 0.59%w/v, respectively. Hydrolysis was performed at 40, 50 and 60 °C for 4, 8 and 12 hours and the results were checked by the measurement of the reducing sugar content.

4. It was found that in the case of the enzymes under investigation 3% was the most appropriate of the three applied concentrations for the hydrolysis of the liquid phase of the suspensions; the addition of 10% of enzyme caused no significant rise in the quantity of the reducing sugars as compared to that obtained with 3%.

5. Under these experimental conditions, with an enzyme concentration of 3% related to the sawdust, the reducing sugar content of the liquid phase of sawdust suspensions is about doubled in the first 8 hours of enzymatic hydrolysis and trebled in 12 hours (Table 3).

6. With the exception of two, the investigated enzyme preparations hydrolyzed the filtrates of the pretreated sawdust suspensions at 40°, 50° and 60 °C, at practically the same rate and to the same degree. At 50 °C the Merck enzyme produced significantly more, at 60 °C the enzyme Meicelase P significantly less reducing sugar than at 40 °C. Consequently, but for the Merck enzyme 40 °C appears to be the most advantageous of the investigated temperatures (Tables 4 and 5).

7. The oligo- and polysaccharides of the sawdust suspension slurry are far more difficult to hydrolyze than those of the liquid phase: at 40 °C the Merck, Chinoin and Serva preparations failed to bring about a degradation of the slurry to reducing sugars even when applied in 10% concentration for 8 hours and only the Meicelase P enzyme appeared to be effective in a concentration of 3%: in contrast to the 116% of reducing substance produced (with reference to the initial value) from the liquid phase, not more than 28% was produced from the slurry (Table 3). The slurry hydrolyzes more readily at 50 °C than at 40 °C (Table 4).

8. There is no relationship between the enzyme concentrations of the preparations as measured on cellulose powder or filter paper substrate and their

effect on the oligo- and polysaccharides of the sawdust hydrolyzate. In the liquid phase the preparations of *Aspergillus* origin were found to be the most active and among them the best results were obtained with Serva cellulase which in a quantity of 1 U (determined on cellulose powder) produced at 40 °C in 8 hours 31.6 μg of reducing matter (expressed in glucose) (Table 7). Under similar conditions the poorest preparation, Sigma Hemicellulase of *Rhizopus* origin, produced 0.8 μg, Meicelase P of *Trichoderma* origin, which is the most active with respect to the oligo- and polysaccharides of the slurry, and the Merck enzyme with the highest activity on cellulose powder, gave 2.7 and 2.5 μg, respectively, of reducing substance. One unit quantity of Meicelase P liberated under similar conditions 0.6 μg of reducing substance from the slurry.

9. In the course of the preliminary experiments the highest reducing substance content, namely 1.65 g per 100 ml, was achieved in the liquid phase of the sulphuric acid prehydrolyzed sawdust suspension by using 3% (related to dry sawdust) of the Merck preparation which had the highest enzyme concentration and performing the enzymatic treatment at 40 °C for 12 hours. This value corresponds to 26.5% of reducing substance related to dry sawdust and is thus better than the result cited in the literature for a similar procedure applied to pine sawdust (11%). The efficiency of enzymatic treatment is still moderate and better results might be expected from the application of appropriately chosen enzyme combinations.

The degradation of cellulose containing industrial and agricultural wastes into low molecular weight digestible sugars is, from the aspect of nutrition and forage, an important potential field of application for cellulolytic enzyme preparations of microbial origin.

So far mainly acid hydrolysis was used to achieve this objective (ANON, 1970). The common drawback of the methods based on acid hydrolysis is the need for expensive acid resistant and occasionally even pressure resistant equipment. It appeared therefore justified to investigate how far the cellulolytic enzyme preparations of microbial origin which have been marketed in the last twenty years, might be applicable to this purpose.

Research related to the application of cellulolytic enzyme preparations has been conducted hitherto with the primary aim to make the starch and protein containing nutrients more readily available, more easily digestible by the degradation of the cellulose and hemicellulose containing cell wall (IMAI & KURODA, 1966; TOYAMA et al., 1966; YASUMATSU et al., 1966; HACHIGA & HAYASHI, 1966; PÁRKÁNY, 1968; EGYED et al., 1969; EGYED & SZABÓ, 1969; ÜRMÖSSY, 1969). There is, however, but scanty reference to the enzymatic hydrolysis of cellulose, i.e. of wood even in recent literature.

Experiments have been carried out to prepare glucose and xylose containing liquors from sawdust by means of combined chemical and enzymatic treatment (DE PADILLA & HOSKINS, 1968). Conifer sawdust was washed with distilled water, finely ground in the laboratory mill and prehydrolyzed with sulphuric acid or sodium hydroxide at 50 °C for 1 to 3 hours and neutralized. After repeated washing with water the pH was adjusted to 5 and the suspension reacted with some cellulolytic enzyme preparation for 1 to 3 hours. The enzyme was then inactivated by keeping the reaction mixture at 80 °C for 15 minutes. The weight of sulphuric acid was 3 to 5 times, its volume 20 times that of the

sawdust, the concentration of sodium hydroxide was 1 to 1.5%, that of the enzyme 1.25% of the sawdust. The best results were obtained by using the highest acid concentration (25%) and 3 hours acid and 3 hours enzymatic treatment, when at the end of the enzymatic treatment 14 to 17% of the cellulose had gone into solution. Sugar yield was 10—11% of the sawdust, the glucose to xylose ratio was about 1 : 1.5.

When studying the bond between lignin and cellulose in pine, swelling in 2 N NaOH and enzymatic treatment were applied alternately (PEW & WEYNA, 1962). In this way it was possible to bring 80% of the carbohydrate into solution. When chemical and enzymatic treatment was preceded by disintegration (10 minutes in the vibro-mill) 95% of the carbohydrate was found in the solution, and when mechanical disintegration was continued for 8 hours chemical treatment had become superfluous since with the enzyme alone 96% of the carbohydrate had been hydrolyzed. Similar results were obtained with poplar. In agreement with these results it was found that when wood-pulp was subjected to enzymatic hydrolysis the results greatly depended on the structure of the pulp (OGIWARA & ARAI, 1969).

Lignin content has a decisive role in the hydrolysis of plant fibres: the higher the lignin content the more resistant the material will be to hydrolysis (OLSON et al., 1937; FULLER & NORMAN, 1943). The inhibitory role of lignin is also indicated by the results obtained in similar experiments with cellulose carried out parallel to the acid-enzymatic hydrolysis of sawdust: with a lower acid concentration (20%) and in a shorter period of time (1 hour) about 21% of the former hydrolyzed, in contrast to the maximum of 17% that could be achieved with sawdust (DE PADILLA & HOSKINS, 1968). In the case of cellulose a highly concentrated hydrolyzate of 30% glucose content could be prepared by high temperature (220 °C) mechanical comminution for 30 minutes, followed by enzymatic hydrolysis (KATZ & REESE, 1968).

Thus, on the basis of literary data, the enzymatic hydrolysis of sawdust depends on the following factors:

— grain size of the sawdust;

— lignin content, i.e. the bond strength between lignin and cellulose;

— efficiency of chemical pretreatment (concentration of the acid or alkali, duration and temperature of treatment).

In addition, the composition of the enzymatic preparation is, of course, also of great importance.

From these research results and from the aspect of a realizable technology it seemed expedient to combine the enzymatic hydrolysis of sawdust with chemical pretreatment. A report shall be given in the following paragraphs on our preliminary experiments concerning the acid-enzymatic hydrolysis of poplar sawdust.

1. Materials and methods

1.1 Materials

1.1.1. The sawdust. In the experiments air dry poplar sawdust was used the moisture content of which was determined prior to each series of experiments by oven drying a known quantity at 105 °C to constant weight. The grain size distribution of the sawdust was established by sieve analysis. The results were:

Below 0.39 mm	13.56 %
0.40—0.62 mm	16.80 %
0.63—0.79 mm	13.36 %
0.80—0.99 mm	13.60 %
1.00—1.59 mm	27.80 %
1.60—2.49 mm	11.24 %
above 2.50 mm	3.64 %
	100.00 %

1.1.2. Acid pretreated sawdust suspensions. The sawdust was pretreated with sulphuric acid at the Department for Chemical Engineering of the Eötvös Loránd University of Sciences. On the basis of preliminary experiments these hydrolyzates neutralized with concentrated ammonium hydroxide were chosen for further processing in which the reducing sugar content did not exceed 10 % of the dry matter of sawdust, since the reducing substance content of sawdust suspensions which had been pre-hydrolyzed to a higher degree showed no substantial change when subjected to enzymatic treatment.

The characteristics of the samples were: pH 6.8 to 8.7, dry sawdust content 5.8 g per 100 ml, reducing sugar content in the filtrate of the slurry containing suspensions 0.31 to 0.49 g per 100 ml, which corresponds to 5 to 8 % related to the dry matter content of the sawdust.

1.1.3. Enzyme preparations. Seven different types of commercial cellulase and one hemicellulase preparation of microbial origin were used in the experiments (Table 1).

1.2. Methods

1.2.1. Pretreatment. Preliminary experiments confirmed that no hydrolysis occurred in the aqueous or buffered suspensions of sawdust when treated with the enzyme preparations listed in Table 1. This holds as well for sawdust suspensions kept at 133 °C for 1 hour prior to enzymatic treatment (VÁMOS et al., 1970). Consequently, in the following experiments the sawdust was treated in moist carbon dioxide atmosphere or with sulphuric acid prior to enzymatic hydrolysis.

Table 1

Parameters of the enzyme preparations used in the enzymatic hydrolysis of sawdust

Name of preparation	Manufacturing firm	Origin of preparation (producing micro-organism)	Enzyme concentration of preparation, U/mg*	
			on cellulose powder substrate	on filter paper substrate
Cellulase	Pharmaceutical and Chemical Works Chinoin, Budapest	Not known	510 ± 4	175 ± 7
Meicelase P	Meiji Seika Kaisha Ltd , Tokyo	*Trichoderma viride*	704 ± 30	188 ± 10
Merck Cellulase	E. Merck AG., Darmstadt	Not known	955 ± 35	230 ± 15
Serva Cellulase	Serva Entwicklungslabor, Heidelberg	*Aspergillus niger*	93 ± 5	93 ± 5
Sigma Cellulase Type I	Sigma Chemical Company, St. Louis, Mo., U.S.A.	*Aspergillus niger*	223 ± 3	136 ± 9
Sigma Cellulase Type II	Sigma Chemical Company, St. Louis, Mo., U.S.A.	*Aspergillus niger*	160 ± 2	106 ± 3
Sigma Cellulase Type III	Sigma Chemical Company, St. Louis, Mo., U.S.A.	*Rhizopus species*	176 ± 12	6 ± 2
Sigma Hemicellulase	Sigma Chemical Company, St. Louis, Mo., U.S.A.	*Rhizopus species*	197 ± 18	51 ± 6

* The preparation has a unit enzyme concentration if 1 mg of it
a) liberates 1 μg of glucose from a 1% suspension of the Macherey and Nagel MN 300 cellulose powder in a medium of pH 5, at 40 °C in 4 hours;
b) liberates 1 μg of glucose from a 1% suspension of the Macherey and Nagel MN 640 filter paper, in a medium of pH 5, at 40 °C in 60 minutes

1.2.1.1. Pretreatment in carbon dioxide atmosphere. 2000 ml of a 10% sawdust suspension in tap water was treated at 130 °C and 12 kp/cm² CO_2 gauge pressure for 2 hours. After cooling the pH of the suspension was adjusted with NaOH to 5.0, diluted to twice the initial volume and hydrolyzed with the enzyme preparation.

1.2.1.2. Pretreatment with sulphuric acid. Sawdust was pretreated with sulphuric acid — as already mentioned — at the Department of Chemical Engineering of the Eötvös Loránd University of Sciences in the following

manner (ANON, 1970): 5.0 g of air dry sawdust of a grain size below 1.00 mm and a dry matter content of 4.62 g was mixed for 5 minutes with 4.7 ml (7.85 g) of 75 w% sulphuric acid in a mortar heated to 55 °C and after the addition of 20 to 40 ml of water heating was continued on a water bath for 15 to 60 minutes. The pH of the hydrolysis liquors and suspensions was adjusted prior to enzymatic hydrolysis to 5 by the addition of NaOH pellets.

1.2.2. Enzymatic hydrolysis. The various sawdust suspensions and hydrolysis liquors were enzymatically hydrolyzed in 100 and 200 ml beakers in an 8 position water-thermostat provided with 180 rpm stirrers. At the end of hydrolysis the samples were placed into a —20 °C refrigerator to stop the enzyme reaction and to prevent the microbial degradation of the sugar containing liquors prior to analysis. Prior to analysis, to make up for evaporation losses, the volumes of the samples were made up with distilled water to the initial value.

1.2.2.1. Hydrolysis of sawdust suspensions pretreated in carbon dioxide atmosphere. One hundred millilitres of the suspensions pretreated in the manner described in par. 1.2.1.1 were incubated with 0, 1.0 and 3.0% (calculated in per cent of the dry sawdust) of cellulase preparations of Merck and Chinoin, respectively, at 40 °C for 3 and 6 hours, filtered and the reducing substance content determined in an aliquot part of the filtrate.

1.2.2.2. Hydrolysis of sawdust samples pretreated with sulphuric acid. The pH of the slurry containing prehydrolyzates prepared at the Department of Chemical Engineering of the Eötvös Loránd University of Sciences, was adjusted to 4.7—5.0 and the slurry separated from the solution by means of centrifuging. The solution and the slurry which was repeatedly centrifuged after washing with distilled water and made up to the initial volume of the suspension with pH 5.1 acetate buffer (0.05 M), were made to react separately with the enzyme. First hydrolysis was performed with 0, 1, 3 and 10% enzyme concentrations related to the dry sawdust content of the suspensions and later, since with 10% enzyme the results were not significantly better, with 1 and 3% enzyme concentrations, at 40°, 50° and 60 °C for 4, 8 and 12 hours. Some of the lower activity enzyme preparations were used in 3 and 10% concentrations only.

In order to observe any spontaneous hydrolysis of the sulphuric acid pretreated sawdust suspensions parallel to every enzymatic hydrolysis experiment, experiments were carried out under identical conditions with so-called "control" samples which contained no enzyme.

1.2.3. Determination of the enzyme concentration of enzyme preparations. The enzyme concentrations of the preparations were determined on cellulose and filter paper substrates at pH 5.0 and 40 °C using reaction times of 1 and 4 hours in 5 parallel experiments for each preparation (ÜRMÖSSY, 1968; NIWA & KAWAMURA, 1965). The amount of the glucose formed was determined

according to SOMOGYI (1952). The preparation has a unit enzyme concentration if 1 mg of it:

a) liberates from a 1% suspension of the Macherey and Nagel MN 300 cellulose powder 1 μg of glucose at pH 5 and 40 °C in 4 hours;

b) liberates from a 1% suspension of the Macherey and Nagel MN 640 filter paper 1 μg of glucose at pH 5 and 40 °C in 60 minutes.

1.2.4. Determination of the reducing substance content. The efficiency of both pretreatment and enzymatic hydrolysis was determined from the reducing substance content. Just as for the determination of enzyme concentration, here too, SOMOGYI's method (1952) was used. Prior to the determination of sugar the proteins were removed from the liquors by means of a phosphotungstic acid solution. The reducing substance content was expressed in glucose. In each experiment three parallel analyses were performed for the determination of the reducing substance content. In this way, in the concentration range between 5 and 30 mg per ml, 96.7 $\pm$ 8.3% of the initial glucose was recovered; the variation coefficient of the parallel determinations was 2.4%

2. Results

2.1. Enzymatic hydrolysis of sawdust suspensions pretreated in carbon dioxide atmosphere

The sawdust suspensions pretreated according to the method described in par. 1.2.1.1 were further hydrolyzed with enzyme preparations as described in par. 1.2.2.1. The reducing substance contents formed during pretreatment and enzymatic hydrolysis are presented in Table 2.

Table 2

Changes in the reducing substance content of sawdust suspensions pretreated at 12 kp/cm² CO_2 gauge pressure and 130 °C, as a result of enzymatic hydrolysis

Reducing substance content of suspension after CO_2 treatment		Name of enzyme preparation											
		Merck Cellulase						Chinoin Cellulase					
		Enzyme concentration related to sawdust		Duration of enzymatic treatment, hours				Enzyme concentration related to sawdust		Duration of enzymatic treatment, hours			
				3		6				3		6	
				Reducing substance after treatment						Reducing substance after treatment			
%w/v	s*	w%	U/g**	$\bar{X}$ %	s*	$\bar{X}$ %	s*	w%	U/g**	$\bar{X}$ %	s*	$\bar{X}$ %	s*
0.33	0.02	1	9 550	266	12.1	306	3.03	1	5 100	142	9.1	160	12.05
0.33	0.02	3	28.650	333	3.03	348	9.1	3	15 300	169	3.02	190	3.1

s* = standard deviation
** = in cellulolytic units measured on cellulose powder
$\bar{X}$ = mean value of experimental data related to the reducing substance content after pretreatment

It appears from the Table that at 12 kp/cm^2 CO_2 gauge pressure and 130 °C temperature pretreatment with carbon dioxide results in the formation of 0.33% reducing substance in the sawdust suspension. With reference to the dry matter content of the sawdust this value is 7%. Enzymatic treatment causes a rise in reducing substance content. The maximum reducing matter content which was achieved by the addition of 3% of the Merck enzyme in 6 hours was 26% of the dry matter content of the sawdust.

The Merck cellulase preparation has a 1.8 times higher cellulolytic activity than Chinoin's enzyme. This ratio appeared in the effect of the preparations, too.

In the case of a 1% enzyme concentration the difference between the actions of the two enzymes is after a 3 hours incubation period highly significant ($P < 0.01$). In the case of 3% enzyme concentration the difference in the efficiency of the two enzyme preparations during a six hours reaction period was very highly significant ($P \ll 0.01$). The differences between the results obtained with the two concentrations of the Chinoin and Merck preparations were also very highly significant (in both cases $P \ll 0.01$).

2.2. Enzymatic hydrolysis of sawdust suspensions pretreated with sulphuric acid

2.2.1. Effect of enzyme concentration and of the duration of hydrolysis. The effects of 3 and 10% and in some cases of 1% enzyme concentrations (related to dry sawdust), on the clear filtrates of pretreated liquors and on slurries suspended in buffer at 40 °C were compared after 4 and 8 hours, and in the case of some preparations also after 12 hours incubation periods. The results are summed up in Table 3.

In order to facilitate comparison in this Table, like in the following Tables, the reducing substance content measured at the end of enzymatic treatment is expressed in percentage of the mean value of reducing substance content formed by pretreatment.

In the case of Merck, Chinoin, Serva and Meicelase P enzymes there is no significant difference in the reducing substance content achieved by the addition of 1, 3 and 10% of enzyme, respectively, after 4 hours hydrolysis.

In 8 hours significantly more reducing substance was formed when 3% of the Merck preparation was added than with 10% of the same preparation, while the difference in reducing substance was highly significant in favour of the experiment with 3% of enzyme compared to the experiment with 1%.

With the Chinoin and Serva preparations there was no significant difference in the reducing substance content after 8 hours treatment with 3 or 10% of the enzyme.

Table 3

*Changes in the reducing substance content of sulphuric acid pretreated sawdust suspensions and filtrates on treatment with various cellulase enzyme preparations at 40 °C**

Name of enzyme preparation	Reducing substance content in the pretreated initial liquor %		Quantity of added enzyme in activity units per 1 ml of liquor or slurry suspension		Quantity of added enzyme in percentage of the sawdust	Duration of enzymatic hydrolysis, hours									
						4				8				12	
						L		Z		L		Z		L	
	$\bar{x}$	s	A	B		$\overline{X}$	s	$\overline{X}$	s	$\overline{X}$	s	$\overline{X}$	s	$\overline{X}$	s
Without enzyme addition	0.39	0.11	0	0	0	101.1	4.4	101.1	4.4	103.8	4.4	103.8	4.4	114.7	11.2
Merck	0.47	0.16	577	139	1	154.3	23.9	154.3	23.9	168	26.5	168.0	26.5	242.5	47.5
	0.49	0.15	1730	417	3	166.3	28.3	166.3	28.3	189	23.0	189.0	23.0	332.5	59.5
	0.47	0.03	5770	1390	10	151.5	1.5	151.5	1.5	157	7.0	157.0	7.0	—	—
Chinoin	0.47	0.16	309	106	1	144.3	41.5	144.3	41.5	155.3	33.7	155.0	33.7	217.0	46.0
	0.49	0.15	930	317	3	177.0	26.3	177.0	26.3	205	34.8	205.0	34.8	286.5	8.5
	0.47	0.03	3090	1060	10	134.5	1.5	134.5	1.5	158.5	11.5	158.5	11.5	—	—
Serva	0.47	0.16	56	56	1	165.0	57.5	165.0	57.5	171.3	41.0	171.3	41.0	209.5	46.5
	0.49	0.15	169	169	3	184.3	66.5	184.3	66.5	213.6	51.3	213.6	51.3	308.0	43.0
	0.47	0.03	560	560	10	130.5	3.5	130.5	3.5	154	8.0	154.0	8.0	—	—
Meicelase P	0.35	0.09	426	114	1	161.3	15.0	172.0	25.0	186.6	6.5	204.5	4.5	—	—
	0.30	0.00	1276	341	3	173.3	42.9	192.3	52.5	216.6	35.0	244.3	39.4	—	—
Sigma I	0.35	0.08	405	247	3	150.0	25.7	150.0	25.7	184.6	40.9	184.6	40.9	—	—
	0.31	0.00	1350	820	10	200.0	10.0	220.5	8.5	209.5	16.5	232.0	20.0	—	—
Sigma II	0.35	0.08	290	192	3	145.3	44.2	145.3	44.2	207.6	40.9	207.6	40.9	—	—
	0.31	0.00	965	640	10	150.0	47.0	177.5	46.5	217.0	33.5	243.5	35.0	—	—
Sigma III	0.35	0.08	318	11	3	104.0	27.5	104.0	27.5	123.6	29.5	123.6	29.5	—	—
	0.31	0.00	1065	35	10	138.5	16.5	188.5	18.5	148.5	3.5	193.5	6.5	—	—
Sigma hemicellulase	0.35	0.08	357	93	3	91.3	20.1	91.3	20.1	109.0	4.5	109.0	4.5	—	—
	0.31	0.00	1190	310	10	110.0	13.0	168.0	13.0	132.0	29.0	179.0	24.0	—	—

* = reducing substance content in % of the reducing substance content of the pretreated liquor, or suspension. L = value measured in the liquor after separation from the sawdust. Z = value measured in the liquor + value obtained in the slurry suspended in buffer. s = standard deviation. $\bar{x}$ = mean value of measurements. $\overline{X}$ = mean value of measurements related to the reducing substance content of the acid prehydrolyzate. A = activity on cellulose powder. B = activity on filter paper suspension.

Neither was there a significant difference in the reducing substance content formed in the liquor after 8 hours treatment with 3 and 10% of the Sigma enzyme. If, however, we consider that as a result of the addition of 10% enzyme an increase in reducing substance content can be measured in the slurry too, then with the exception of the preparation Sigma II the addition of 10% enzyme was significantly more effective than the addition of 3%.

Despite the fact that the addition of the same percentage concentration corresponded to greatly different enzyme concentrations when using different enzyme preparations, there was no significant difference in their effect after 8 hours: with 3% of Merck enzyme 189% ($\pm$23.02), with 3% Chinoin cellulase 205% ($\pm$34.81) and with the Serva enzyme 213% ($\pm$51.37) reducing substance content was measured (in all three cases $P > 0.05$).

The reducing substance content of the liquor pretreated with sulphuric acid was 0.49 %w/v ($\pm$0.15). This value when related to dry sawdust, corresponded to 8.0%. After 8 hours hydrolysis the reducing substance content obtained with the Merck enzyme corresponded to 15.1%, with the Chinoin enzyme to 16.4% and with the Serva enzyme to 17.0% of the dry sawdust.

Under similar conditions the addition of Meicelase P enzyme resulted in 216.6% of reducing substance related to the initial liquor and, accounting for the degradation of the slurry, to 244.3% reducing substance content, but — as the reducing substance content of the initial suspension was lower (0.30 %w/v) — the final results in %w/v and related to sawdust were lower: namely 0.65 %w/v or 10.5%. With application of Sigma enzymes 109 to 207.6% values were measured relative to the initial 0.37% reducing substance content (Table 3).

With Merck, Chinoin and Serva enzymes 12 hours hydrolyses were also carried out. The reducing substance content obtained in this manner was significantly higher than the values measured after 8 hours (in all three cases $P < 0.05$).

As an effect of the addition of 3% enzyme preparation related to the dry sawdust and a hydrolysis period of 12 hours the 0.49 %w/v reducing substance content of the initial liquors (pretreated with sulphuric acid) rose in the case of the Merck enzyme to 1.63 per 100 ml (332.5%), or related to sawdust to 26.5%, in the case of Chinoin and Serva enzymes to 1.34 %w/v (286.5%) and 1.44 %w/v (308%), respectively. These values correspond to 21.7 and 23.4% of the dry matter content of sawdust.

Compared to the values obtained after 8 hours hydrolysis, the increase is in the case of Merck enzyme 75%, in the case of Chinoin cellulase 40% and of Serva enzyme 44%.

2.2.2. Effect of the temperature of hydrolysis. It appears from Fig. 1 that the reducing substance content of the control samples which contain no enzyme

changes too, provided they are kept at a higher temperature for some length of time.

Changes in the sugar content of the control samples as compared to the initial values are not significant in 8 hours when the samples are kept at 40 °C. At 50 °C the reducing substance content rises to 142% of the initial value (±4.36). This value differs very highly significantly from the values obtained

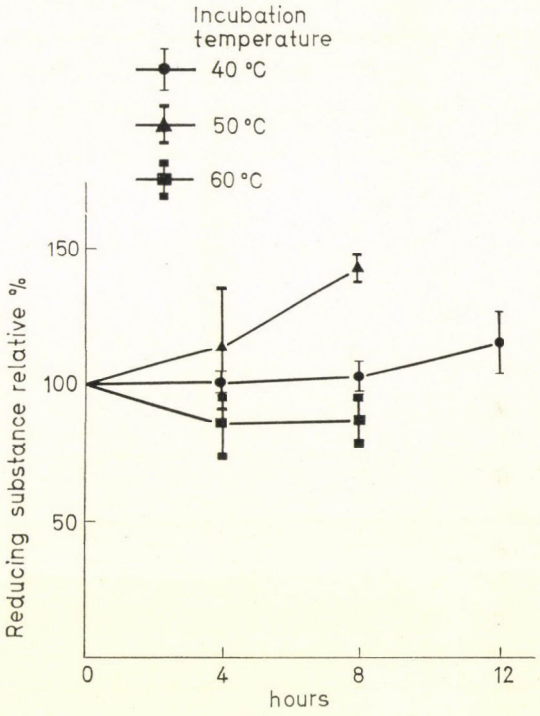

Fig. 1. Changes in the reducing substance content of sulphuric acid pretreated sawdust suspensions, vs. time, without the presence of enzyme, at various temperatures. The perpendicular line represents the value of the standard deviation (s) N = 3

at 40 °C in 8 hours ($P \ll 0.01$). At 60 °C 87.3% of the initial value is recovered (±11.55). The deviation of the latter value from that obtained at 40 °C is significant ($P < 0.05$).

The results indicate the lack of spontaneous hydrolysis of oligosaccharides as well as sugar degradation at 40 °C. At 50 °C spontaneous hydrolysis is significant, while at 60 °C sugar degradation dominates.

The effect of temperature on enzymatic hydrolysis was investigated in the presence of 3% of enzyme and reaction periods of 4 and 8 hours at 40°, 50° and 60 °C, since most cellulase preparations reach their maximum activity between 40 and 60 °C.

The results of the experiments are presented in Tables 4 and 5.

Table 4

*Changes in the reducing substance content of sulphuric acid pretreated sawdust suspensions and filtrates on treatment with various cellulase enzyme preparations at 50 °C**

Name of enzyme preparation	Reducing substance content in the pretreated initial liquor %		Quantity of added enzyme in activity units per 1 ml of liquor or slurry suspension		Quantity of added enzyme in percentage of the sawdust	Duration of enzymatic hydrolysis, hours							
						4				8			
						L		Z		L		Z	
	$\bar{x}$	s	A	B		$\overline{X}$	s	$\overline{X}$	s	$\overline{X}$	s	$\overline{X}$	s
Without enzyme addition	0.39	0.11	0	0	0	114.6	21.4	114.6	21.4	142	4.4	142.0	4.4
Merck	0.49	0.15	1730	417	3	224.6	18.3	241.6	18.3	238.3	19.8	263.3	19.8
Chinoin	0.49	0.15	930	317	3	176.6	39.2	192.6	39.2	208.0	41.9	228.0	41.9
Meicelase P	0.30	0.00	1276	341	3	112.0	36.3	—	—	153.0	43.3	—	—
Sigma I	0.36	0.08	405	247	3	177.6	41.8	195.6	41.8	186.0	104.8	204.0	104.8
Sigma II	0.36	0.08	290	192	3	154.3	28.5	171.3	28.5	188.3	46.6	213.3	46.6
Sigma III	0.36	0.08	318	11	3	138.3	27.5	160.3	27.9	158.3	24.8	178.3	24.8
Sigma hemicellulase	0.36	0.08	357	93	3	124.0	55.5	150.0	55.5	161.0	36.7	188.0	36.7

* = reducing substance content in % of the reducing substance content of the pretreated liquor or suspension
L = value measured in the liquor after separation from the sawdust
Z = value measured in the liquor + value obtained in the slurry suspended in buffer
s = standard deviation
$\bar{x}$ = mean value of measurements
$\overline{X}$ = mean value of measurements related to the reducing substance content of the acid prehydrolyzate
A = activity on cellulose powder
B = activity on filter paper suspension

Table 5

*Changes in the reducing substance content of sulphuric acid pretreated sawdust suspensions and filtrates on treatment with various cellulase enzyme preparations at 60 °C**

Name of enzyme preparation	Reducing substance content in the pretreated initial liquor %		Quantity of added enzyme in activity units per 1 ml of liquor or slurry suspension		Quantity of added enzyme in percentage of the sawdust	Duration of enzymatic hydrolysis, hours							
						4				8			
						L		Z		L		Z	
	$\bar{x}$	s	A	B		$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s
Without enzyme addition	0.39	0.11	0	0	0	85.3	12.7	85.3	12.7	87.3	11.6	87.3	11.6
Merck	0.49	0.15	1730	417	3	191.6	34.4	208.6	34.4	209.6	18.5	215.6	18.5
Chinoin	0.49	0.15	930	317	3	174	19.9	189	19.9	174	46.1	192	46.1
Meicelase P	0.30	0.00	1276	341	3	135.6	3.7	—	—	115.6	11.7	—	—
Sigma I	0.35	0.08	405	247	3	181	35.5	201	35.5	161.6	72.4	183.6	72.4
Sigma II	0.35	0.08	290	192	3	186.6	55.2	204.6	55.2	169.1	29.6	194.6	29.6
Sigma III	0.35	0.06	318	11	3	145.6	42.7	174.6	42.7	156	31.1	188	31.1
Sigma hemicellulase	0.35	0.08	357	93	3	100.3	46.7	120.3	46.7	108	51.9	128	51.9

* = reducing substance content in % of the reducing substance content of the pretreated liquor, or suspension
L = value measured in the liquor after separation from the sawdust
Z = value measured in the liquor + value obtained in the slurry suspended in buffer
s = standard deviation
$\bar{x}$ = mean value of measurements
$\bar{X}$ = mean value of measurements related to the reducing substance content of the acid prehydrolyzate
A = activity on cellulose powder
B = activity on filter paper suspension

In the case of the Merck enzyme, of the three temperatures 50 °C is significantly ($P < 0.05$) the optimum. With the Chinoin and the four types of Sigma preparations the differences are not significant.

2.2.3. Effect of the various enzymes on the slurry phase of the pretreated suspensions. At 40 °C no detectable quantity of reducing sugar was formed on treatment with Merck, Chinoin and Serva cellulases, respectively, in 8 and 12 hours. When applied in a concentration of 3% Meicelase P and in 10% concentrations the four types of Sigma enzymes had an effect on the slurry, the dissolved amount of reducing substance rose by 20 to 40% of the initial value, depending on incubation period and enzyme concentration. At 50° and 60 °C the addition of 3% of Merck and Chinoin enzymes led as well to the formation of reducing sugar in the slurry (the Serva and Meicelase P enzymes were not tested at these temperatures). The effect of Sigma enzymes was manifest also at higher temperatures in an about 20% increase of the reducing sugar content.

In columns Z of Tables 3, 4 and 5 the overall reducing substance content in the liquor and slurry are given.

3. Conclusions

3.1. Role of the pretreatment of sawdust

According to our experiments both treatment at high pressure and high temperature with weak acid (CO_2) and treatment at a relatively low temperature (55 °C) with strong mineral acid makes the cellulose and hemicellulose content of sawdust accessible to enzymes.

The maximum value which can be achieved by means of enzymatic treatment is 26.5% reducing substance related to dry sawdust (prehydrolysis with sulphuric acid and treatment with 3% Merck enzyme related to dry sawdust, at 40 °C, in 12 hours). Compared to the best results which DE PADILLA & HOSKINS (1968) achieved with their acid-enzymatic method, the above value is more than twice as high, but is still far below the theoretical value. The total cellulose and hemicellulose content of poplar is about 77% (NIKITIN, 1955) which, of course, varies according to variety and geographical region, etc.

Carbon dioxide digestion under pressure resulted in the formation of about 7% of reducing substance related to dry sawdust, thus the yield is of roughly the same order as in the case of pretreatment with sulphuric acid. The reducing substance content of samples pretreated with carbon dioxide rose to nearly 350% of the initial value after treatment with 3% of Merck enzyme (for dry sawdust) at 40 °C for 6 hours, while under similar conditions the reducing substance content of sulphuric acid pretreated samples rose only to 190% in 8 hours, corresponding to a 1.15 and 0.92 g per 100 ml reducing

substance content, respectively. Thus pretreatment with carbon dioxide appears to be more favourable from the aspect of the enzymatic hydrolysis of sawdust. It has, however, the drawback of requiring expensive equipment.

3.2. Role of the parameters of enzymatic hydrolysis

3.2.1. Enzyme concentration. In the majority of our experiments 1 and 3 and occasionally 10% enzyme concentrations were used for the dry sawdust. Related to the liquor or slurry to be hydrolyzed this corresponds to 0.06, 0.18 and 0.59% w/v. With a 3% enzyme concentration generally better results were obtained than with 1%, but the difference was not in proportion to the surplus enzyme quantity. In the case of samples pretreated with sulphuric acid the relative difference between the reducing substance contents achieved with the various concentrations was the higher the longer the period of hydrolysis. After 4 hours hydrolysis there was no significant difference between the reducing substance contents achieved with the three different enzyme concentrations. The ratio between the reducing substance contents brought about by 1 and 3% enzyme concentrations after 6 and 12 hours incubation are shown in Table 6 for the various enzyme preparations and the two types of acid pretreatment.

When 10% of enzyme related to dry sawdust is added, the reducing substance yield is either similar or significantly lower than with 3%. So far we have been unable to find an explanation for this observation.

Experiments with buffered slurry suspensions have shown that there are oligo- and polysaccharides in the slurry which can be hydrolyzed to reducing

Table 6

Ratio of reducing substances formed on the addition of 1 and 3% of various enzyme preparations in the course of the hydrolysis of sawdust suspensions obtained by sulphuric acid and carbon dioxide pretreatments

Name of enzyme preparation	Method of pretreatment	
	Sulphuric acid digestion 12 hours	Carbon dioxide digestion 6 hours
Merck cellulase	1 : 1.6**	1 : 1.2***
Chinoin cellulase	1 : 1.5**	1 : 1.5***
Serva cellulase	1 : 1.9*	

In the case of the values marked with *, ** and *** there was a significant, a highly significant and a very highly significant difference, respectively, between the reducing substance contents obtained with 1 and 3% enzyme concentrations

substances, but these are more difficult to hydrolyze and then only with higher enzyme concentrations and not with all the investigated enzymes.

3.2.2. Duration of enzymatic hydrolysis. In the case of Merck, Chinoin and Serva cellulase preparations the sawdust suspensions which had been pretreated with sulphuric acid were hydrolyzed up to 12 hours. Contrary to expectation the reducing substance content measured after 12 hours showed a sudden rise compared to that after 8 hours in case of all three preparations. On addition of 3% of enzyme related to dry sawdust the reducing substance content of acid prehydrolyzates increased in the first 8 hours by 89, 105 and 114%, respectively, and in the next 4 hours by 143, 81 and 95%. In the 4 hours period between the 8th and 12th hour the increase in reducing substance content was of the same order as in the preceding 8 hours period. This appears to indicate that it might be advisable to continue enzymatic hydrolysis for a longer period of time.

3.2.3. Temperature of enzymatic treatment. Most enzyme preparations gave practically the same results at 40, 50 and 60 °C, with the exception of the Merck enzyme which gave the best result at 50 °C and Meicelase P which gave a significantly poorer result in the filtrate of sawdust suspensions at 60 °C. This last result might be attributed to the instability of this enzyme at 60 °C (MEIJI SEIKA KAISHA, 1967). Thus the formation of reducing substance content observed as the result of complex enzyme reactions, with the exception of the Merck preparation, cannot be accelerated by an increase of the temperature.

This result indicates at the same time that the spontaneous oligo- and polysaccharide degradation taking place in the enzyme-free control samples of the acid hydrolyzates which is the most marked at 50 °C and the decomposition of reducing sugars in these samples which is the most significant at 60 °C will not occur or will occur in a different manner in the presence of enzyme.

It is an interesting fact that the slurry which hydrolyzes less readily than the filtrate of the suspension so that it will resist hydrolysis even in the presence of 10% Merck enzyme at 40 °C, will at 50 °C and with 3% enzyme already give reducing sugars (the change is significant after 8 hours hydrolysis).

3.3. Effect of the quality of enzyme preparations

Of the eight enzyme preparations used in the experiments 3 were of *Aspergillus*, 2 of *Rhizopus*, 1 of *Trichoderma* and 2 of unknown microbial origin. The enzyme concentrations of these preparations, as determined on cellulose powder and filter paper substrates, showed marked differences. On cellulose powder substrate there is a more than tenfold difference between

Serva enzyme and the Merck preparation, representing the lowest and highest enzyme concentrations, respectively. On filter paper substrate the enzyme concentration of the Merck preparation is nearly 40 times higher than that of the Sigma Type III preparation with the lowest enzyme concentration (Table 1). Our results show practically no relationship between the enzyme concentration values determined on the substrates — consisting essentially of pure alpha-cellulose — and the enzyme effect measured on multi-component prehydrolyzed sawdust suspensions (Table 3). When treating other natural substrates, e.g. potato parenchymal cell walls with cellulase preparations it was also observed that the hydrolyzability cannot be brought into correlation with the activity of the enzyme preparation measured on cellulose substrate (RODIONOVA et al., 1970). If, for instance, the reducing substance content formed at 40 °C in 8 hours as an effect of treatment with 3% enzyme related to dry sawdust is compared to the corresponding data in Table 3 and related to 1 activity unit (taking the activity measured on cellulose powder as the basis of comparison) the data presented in Table 7 are obtained.

Table 7

Reducing substance formed under the action of 1 U quantities of the various enzyme preparations on sulphuric acid pretreated sawdust suspension

3% enzyme preparation related to dry sawdust, 40 °C, 8 hours

Name of preparation	Reducing substance in μg formed by the action of 1 U of enzyme*
Merck	2.5
Chinoin	5.6
Serva	31.6
Meicelase P	2.7
Sigma I	7.2
Sigma II	12.8
Sigma III	2.5
Sigma Hemicellulase	0.8

* Activity measured on cellulose powder.

It appears from the Table that with an enzyme quantity of unit activity the highest amount of reducing substance is obtained with the Serva preparation, and then with Sigma II and Sigma I preparations. All three preparations are of *Aspergillus niger* origin. The cellulolytic enzyme complex of *Aspergillus niger* origin does not attack the fibrous cellulose molecules of high degree

of polymerization (WHISTLER & SMART, 1953; IKEDA et al., 1967). Consequently, this enzyme has a low activity on cellulose powder and filter paper substrates, while it causes a marked degradation of the sulphuric acid prehydrolyzed, thus obviously somewhat already depolymerized, cellulose. It is an interesting fact that of these preparations only two, which had been found less active towards the filtrate, attack the slurry at 40 °C, namely Sigma I and II, while Serva does not hydrolyze the slurry.

After the Sigma enzymes comes the enzyme preparation of Chinoin of practically identical activity with Meicelase P and the Merck and Sigma III preparations. Sigma hemicellulase, which is in fact a galactan-splitting preparation, is the weakest.

Meicelase P enzyme of *Trichoderma* origin was found the most suitable for the hydrolysis of the slurry: of the investigated preparations this was the only one which, when applied at 40 °C in a 3% concentration, significantly increased the reducing substance content of the slurry, so that 1 U of the preparation produced 0.6 μg of reducing substance.

The difference between the activities of the enzyme preparations is confirmed by the results of chromatographic tests of the sugar composition of the hydrolyzates. These results will be reported in another communication.

3.4. Efficiency of acid combined enzymatic hydrolysis

In our experiments aqueous suspensions of approximately 5 % w/v poplar sawdust content were processed. The liquor with the highest sugar concentrations was obtained by 12 hours enzymatic hydrolysis of the filtrate of a sulphuric acid prehydrolyzed slurry, containing 0.49 $\pm$ 0.16% of reducing substance, using 3% of Merck cellulase preparation (related to dry sawdust), that is 0.18 g per 100 ml of the filtrate. This highest sugar concentration was 1.63 % w/v and 26.5% of the sawdust was converted into reducing substances. Though this value is higher than the result reported in the literature for the combined acid and enzyme treatment of pine sawdust (DE PADILLA & HOSKINS, 1968), the necessary quantity of enzyme preparation is relatively high: with 1 g of the enzyme preparation 9 g sugar suitable for assimilation by yeast may be produced.

If the entire polysaccharide content of sawdust could be converted into reducing substances, then in the above case aliquor with 3.5 to 4.0% sugar content would be obtained. Though the residual slurry obtained after acid prehydrolysis of sawdust still contains some high molecular unsplitted oligo- and polysaccharides, these latter can be hydolyzed only with difficulty, with a high enzyme input even when using *Aspergillus* enzymes which are the most active towards oligosaccharides or with Merck preparation of high enzyme concentration. Better results may be expected from the combined

application of these enzymes with Meicelase P. of *Trichoderma* origin which has a greater effect on the polysaccharides of the slurry.

In forthcoming communications our experiments aimed at the establishment of the optimum conditions of acid pretreatment (sawdust and acid concentration, temperature, duration), as well as at the hydrolyzability of pretreated sawdust suspensions with enzyme mixtures and at the determination of pretreatment efficiency will be reported.

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EFFECT OF SORBIC ACID ON THE CARBOHYDRATE FERMENTATION AND OLIGOSACCHARIDE SPLITTING ENZYMES OF YEASTS

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The influence of sorbic acid (SA) on the fermentation of five different carbohydrates (glucose, galactose, sucrose, maltose, raffinose) with six yeast species *(Candida claussenii, C. utilis, Procandida albicans, Pc. tropicalis, Saccharomyces carlsbergensis, S. cerevisiae)* has been investigated. The fermentation was inhibited in each case, significant differences were found, however, in the degree of inhibition of fermentation when different carbohydrates were tested with one given species, or when identical carbohydrates were fermented by different yeast species. The SA inhibition of fermentation was found to be stronger generally with the intracellularly splitted oligosaccharides as compared to glucose. On the other hand the degree of inhibition of the extracellularly slpitted oligosaccharides was equal to that of glucose. In the cell free extracts and acetone treated cells of *C. claussenii*, *Pc. albicans* and *S. carlsbergensis*, the SA failed to inhibit both the sucrose and the maltose decomposing enzyme activities. Relying on these findings and considering the steps differing from each other in the metabolism of the carbohydrates studied, the diversity in the degree of SA inhibition suggests, that SA inhibits the transport of certain carbohydrates to different degrees.

Several papers have been published on the explanation of the mechanism of effect of sorbic acid. A part of them deals with the effect of SA demonstrable in various steps of the metabolism of aerobic energy production. Thus SA inhibition was described in reactions connected with coenzyme A (PALLERONI & PRITZ, 1960; HARADA et al., 1968), with certain enzymes of the citric acid cycle (YORK & VAUGHN, 1964; REHM, 1967), and with the terminal oxidation (ANDERSON, 1963). Possible sites of attack of SA were referred to in anaerobic systems as well. WHITAKER (1959) supposed, on the ground of inhibition of the crystalline alcohol dehydrogenase as well as on the fact that this inhibition could be stopped partly by cysteine, SA to be an effective inhibitor of sulphhydryl enzymes. The effect on alcohol dehydrogenase was explained by the addition developed between the reactive $ZnOH^-$ or SH^- groups of the enzyme and the β or δ double-bonds of SA (MARTOADIPROWITO & WHITAKER, 1963).

On the other hand according to AZUKAS et al. (1961) the SA-inhibition of alcoholic fermentation of yeasts might be ascribed to enolase-inhibition, as the SA failed to inhibit the activity of several other enzymes of glycolysis (among others that of alcohol dehydrogenase and aldolase) in cell-free extracts. However, if SA inhibits the enolase what might explain the lack of its effect upon lactic acid bacteria (EMARD & VAUGHN, 1952; COSTILOW et al., 1955;

BELL et al., 1959), the role of enolase being identical in both lactic acid and alcoholic fermentations. AZUKAS (1962) considers the lactic acid dehydrogenase to protect the enolase from the SA effect, as according to his experience the inhibition of enolase by SA can be stopped by adding to it purified lactic acid dehydrogenase. On the other hand SPRINGER and SCHEGK (1965) as well as RASMUSSEN (1967) have demonstrated that SA inhibits also the isolated lactic acid dehydrogenase, furthermore in moulds of the enzymes of glycolysis only in the case of aldolase could significant inhibition be observed.

Considering the contradictions which can be observed mostly in the *in vitro* results, as cited from the literature above, we have found necessary to study the effect of SA on the alcoholic fermentation of yeasts from another viewpoint, in *in vivo* systems.

1. Materials and methods

Potassium sorbate (Farbwerke Hoechst AG) was used for sorbic acid. The carbohydrates applied, checked by chromatography, proved to be free from impurities except maltose, which contained about 8% glucose and 1% maltotriose.

The yeasts studied were the following:

Candida claussenii 460-sect/1961
Candida utilis 287/1964
Procandida albicans 85/1957
Procandida tropicalis 302/1964
Saccharomyces carlsbergensis II/1966
Saccharomyces cerevisiae CXCII/1967

The maintenance and propagation of the strains was performed as described earlier (NOVÁK & DEÁK, 1970). Two methods were used for fermentation experiments. Pepton-water medium was used for the Einhorn-tube tests (LODDER & KREGER VAN RIJ, 1952), with 4% fermenting substrate (NOVÁK, 1967). The inoculation was carried out with 0.1 ml suspension of 10^8/ml cell density adding 0.1 ml yeast extract to the tubes simultaneously. The degree of fermentation was measured with the quantity of gas produced, which could be read directly in ml in the calibrated tube-arms. The rate of fermentation was expressed in units of ml gas/hour as calculated from the values of the linearly rising section of the fermentation curves. The Einhorn-tube tests were performed in three parallels.

The manometric procedure was the other technique used for fermentation examinations and was carried out in the usual way (UMBREIT et al., 1957),

in nitrogen gas atmosphere washed twice through alkaline pyrogallol solution. A detailed description of the composition of the experimental systems used is given with each experiment separately. The rate of fermentation is expressed in units of μl CO_2/mg dry cell material, based on 2 parallel experiments.

The strains for studying the oligosaccharide splitting enzymes were cultivated in Roux flasks on molasses agar (CSILLAG, 1951) incubating them for 48 hours at 26 °C. The cells were washed in physiological salt solution, centrifuged three times (2500 ×g) and suspended repeatedly in distilled water. After this procedure the packed cell mass was cooled to 4 °C.

The method to produce cell-free preparations and acetone treated cells was basically identical with the technique of NOVÁK (1961). To prepare cell-free extracts, the wet cell mass was ground with cooled (4° C) quartz sand. After 10 minutes grinding the cell mass was suspended in 1/15 M pH 5.5 phosphate buffer, so that every ml contained 0.5 g, as calculated for the original wet cell mass. The suspension was subjected to repeated centrifugation (5000 ×g for 30 minutes) till clear supernatant was obtained. For acetone treatment an equal volume of ice-cold acetone was given to the suspension in small quantities, under constant stirring. After centrifugation (2500 ×g for 10 minutes) the supernatant was discarded, and the acetone treatment was repeated. After repeated centrifugation the sediment was dried in glass dishes at room temperature and suspended subsequently in 1/15 M pH 5.5 phosphate buffer as described above.

For measuring the oligosaccharide-decomposing activity, the substrate studied (sucrose or maltose) was given to the cell-free extract or acetone treated cells, in 10% of the volume of the preparation, the final concentration being 2%. The oligosaccharide-decomposing activity was characterized by the quantity of glucose produced from the corresponding oligosaccharide, as a function of time.

To determine the glucose formed, samples of 0.5 ml were taken at predetermined intervals. The enzymes were inactivated by 10 minutes boiling, and the samples were deproteinized by the $ZnSO_4$—$Ba(OH)_2$ method of NELSON (1944). After centrifugation appropriate dilution was made from the aliquot of the supernatant, and the glucose was determined according to the calibration curve, using glucose oxidase reagent ("Glucostat" ultramicro preparation, Worthington Biochem. Corp.). Photometric measuring was carried out in the photoelectric colorimeter, type FEK-M.

To study the effect of SA on the oligosaccharide splitting enzymes, only those species were selected which contained different types of these enzymes. The species selected were the following: *C. claussenii*, a yeast species containing endosaccharase and exomaltase (NOVÁK et al., 1966; TÜSKE, 1967), *Pc. albicans* containing endosaccharase and endomaltase (NOVÁK & ZSOLT, 1963a, b), and *S. carlsbergensis* which splits sucrose with invertase and maltose with endo-

maltase (NOVÁK, 1961; MILLIN, 1963). Three parallels were made with *Pc. albicans* and two with the other species studied.

2. Results

The time courses of fermentation in Einhorn-tube experiments are demonstrated in Fig. 1. It is seen that the rate of fermentation decreases with the increase of SA concentration. The inhibiting dosage response curves of

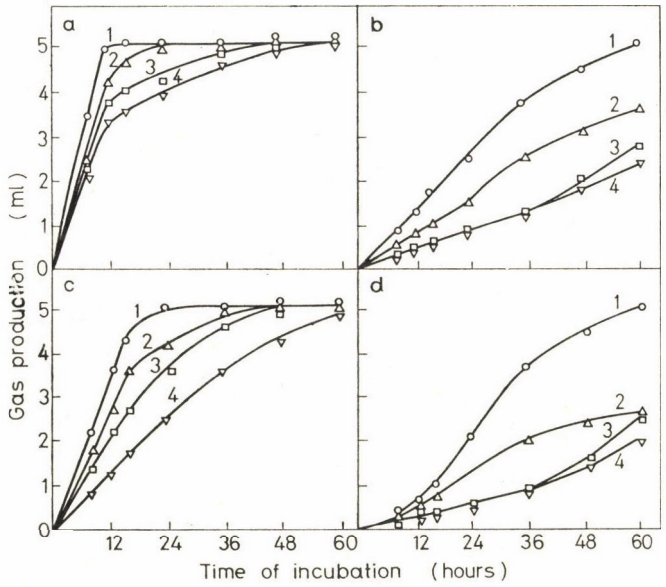

Fig. 1. Effect of sorbic acid on the fermentation of various carbohydrates with *Procandida albicans*. Einhorn-tube fermentation, peptone-water with 4% sugar. a: glucose, b: galactose, c: sucrose, d: maltose, o control, △ 0.18 mg/ml SA, □ 0.38 mg/ml SA, ▽ 0.75 mg/ml SA

SA, however, changed according to both the species and the fermentation substrates (Fig. 2). Striking is the fact that *S. carlsbergensis* proved to be the most resistant to SA inhibition. It is emphasized furthermore, that the degree of inhibition in the case of the two substrates, glucose and maltose, remained unchanged only with the species *C. claussenii*.

Results obtained with Einhorn-tube tests, including every substrate and species studied, are summarized in Table 1. It is shown that as compared with glucose, the SA inhibition of galactose fermentation differed with each species.

The same phenomenon was observed in the case of maltose fermentation except with *C. claussenii*. On the other hand, comparing the fermentation of glucose, sucrose and raffinose greater than 10% deviation in the degree of inhibition could be observed only with *Pc. tropicalis* (in the case of sucrose) and with *S. carlsbergensis* (in the case of raffinose).

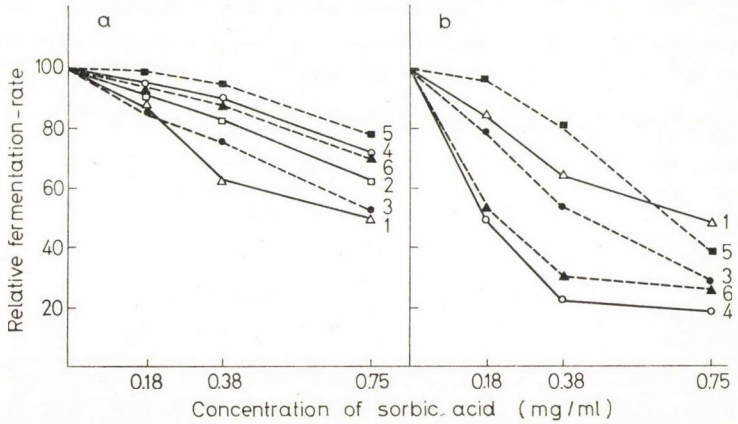

Fig. 2. Changes in the fermentation-inhibiting effect of sorbic acid, as a function of the concentration of the inhibitor, the substrates and the species, a: glucose fermentation, b: maltose fermentation, 1: *C. claussenii*, 2: *C. utilis*, 3: *Pc. albicans*, 4: *Pc. tropicalis*, 5: *S. carlsbergensis*, 6: *S. cerevisiae*

Table 1

Effect of sorbic acid on the fermentation of various carbohydrates

Figures represent the percentage values of the inhibition of fermentation rate, as compared to the control without SA. SA concentration 0.75 mg/ml. Einhorn-tube tests, pepton-water medium, 4% fermentation substrate

Species	Substrate				
	D	G	S	M	R
C. claussenii	50	71	—	52	—
C. utilis	38	—	31	—	43
Pc. albicans	48	—	—	69	—
Pc. tropicalis	30	70	52	80	—
S. carlsbergensis	22	30	22	60	35
S. cerevisiae	31	—	25	72	37

D: glucose
G: galactose
S: sucrose
M: maltose
R: raffinose
—: not fermented by the species

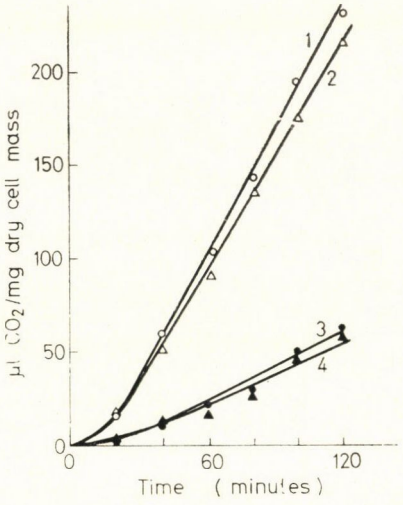

Fig. 3. Effect of sorbic acid on the glucose- and maltose-fermentation of *Candida clausenii*. Manometric tests at 30 °C, in N_2 atmosphere. Content of Warburg vessels: 1.55 mg dry cell mass, 40 mg sugar (○ glucose, △ maltose) and 0.50 mg/ml SA where indicated (black marks) in 2 ml total volume of 1/15 M phosphate buffer, pH 5.5

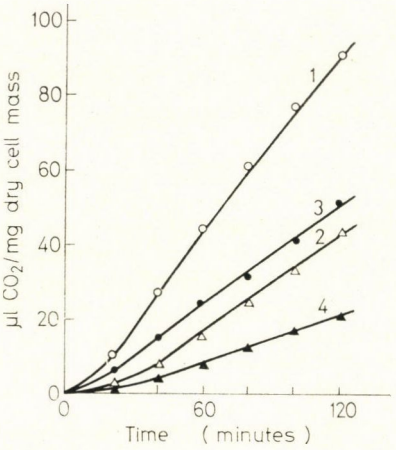

Fig. 4. Effect of sorbic acid on the glucose- and maltose-fermentation of *Procandida albicans*. Manometric experiments at 30 °C, in N_2 atmosphere. Content of Warburg vessels: 1.40 mg dry cell mass, 40 mg sugar (○ glucose, △ maltose), and 0.50 mg/ml SA where indicated (black marks) in 2 ml total volume of 1/15 M phosphate buffer, pH 5.5

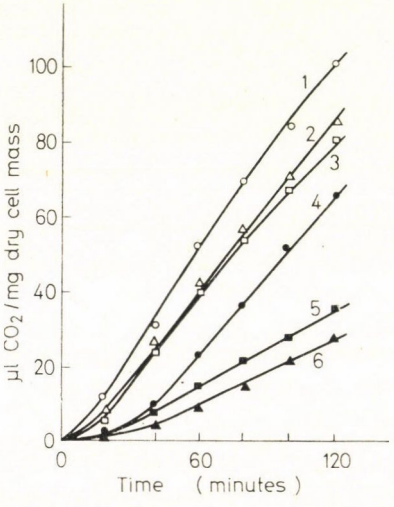

Fig. 5. Effect of sorbic acid on the glucose-, sucrose- and maltose-fermentation of *Procandida tropicalis*. Manometric experiments at 30 °C, in N_2 atmosphere. Content of Warburg vessels: 1.40 mg dry cell mass, 40 mg sugar (○ glucose, △ maltose, □ sucrose), and 0.50 mg/ml SA where indicated (black marks) in 2 ml total volume of 1/15 M phosphate buffer, pH 5.5

Table 2

Effect of sorbic acid on anaerobic CO_2 production

Manometric experiments at 30 °C, in N_2 atmosphere. Content of Warburg vessels, in 2 ml total volume of 1/15 M, pH 5.5 phosphate buffer: 1.0 to 3.0 mg dry cell mass. 40 mg sugar (D: glucose, S: sucrose, M: maltose, R: raffinose), and 0.50 mg/ml SA, where indicated. Values of Q_{CO_2}: μl CO_2/hour × mg dry weight

Species	Substrate	Inhibitor	Q_{CO_2}	Inhibition %
C. claussenii	D	—	115.0	
	D	SA	32.4	71.8
	M	—	109.0	
	M	SA	30.5	72.0
Pc. albicans	D	—	46.3	
	D	SA	25.9	44.0
	M	—	23.2	
	M	SA	10.1	56.3
Pc. tropicalis	D	—	51.3	
	D	SA	33.0	35.8
	S	—	40.3	
	S	SA	18.1	51.3
	M	—	42.7	
	M	SA	14.1	66.9
S. carlsbergensis	D	—	60.0	
	D	SA	54.5	9.0
	S	—	67.8	
	S	SA	62.7	7.6
	M	—	50.3	
	M	SA	33.0	34.5
	R	—	47.8	
	R	SA	43.2	9.5

The results of Einhorn-tube tests were confirmed with manometric examinations. No diversity was found in the degree of SA-inhibition of glucose and maltose fermentation with *C. claussenii* (Fig. 3), while stronger inhibition could be observed in maltose fermentation with *Pc. albicans* (Fig. 4), *Pc. tropicalis* (Fig. 5) and with *S. carlsbergensis* (Fig. 6). In the case of *Pc. tropicalis* the inhibition both of maltose- and of sucrose fermentation was stronger than that of the glucose (Fig. 5), while with *S. carlsbergensis* not only the glucose and sucrose fermentation revealed identical degrees of inhibition by SA, but — in contradiction to the Einhorn test results — the raffinose, too (Fig. 6). The results of manometric examinations are presented in Table 2. The effect of SA (1.5 mg/ml) on the sucrose- i.e. maltose-decomposing activity in the

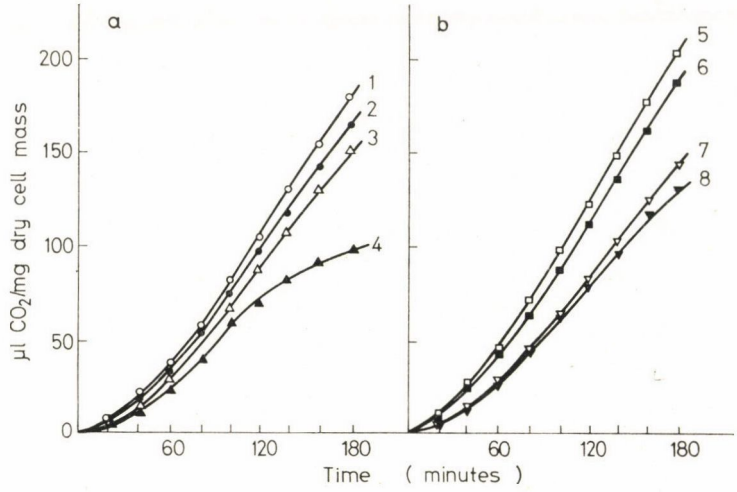

Fig. 6. Effect of sorbic acid on the fermentation of various carbohydrates with *Saccharomyces carlsbergensis*. Manometric experiments at 30 °C in N_2 atmosphere. Content of Warburg vessels: 3.0 mg dry cell mass, 40 mg sugar (○ glucose, △ maltose, □ sucrose, ▽ raffinose), and 0.50 mg/ml SA where indicated (black marks) in 2 ml total volume of 1/15 M phosphate buffer, pH 5.5

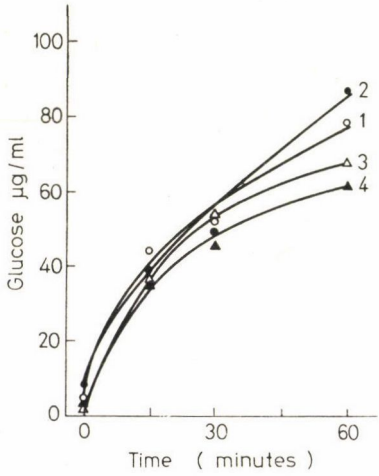

Fig. 7. Effect of sorbic acid on the sucrose- and maltose-decomposing activity of the cell-free extract of *Candida claussenii*. Incubation system: 1.6 ml cell-free extract in 1/15 M phosphate buffer (pH 5.5), 0.2 ml of 20% sucrose (1 and 2) or maltose (3 and 4), further 0.2 ml distilled water (control: 1 and 3), or 0.2 ml of 15 mg/ml SA solution (2 and 4). Incubation at 25 °C. Determination of glucose with glucose-oxidase reagent

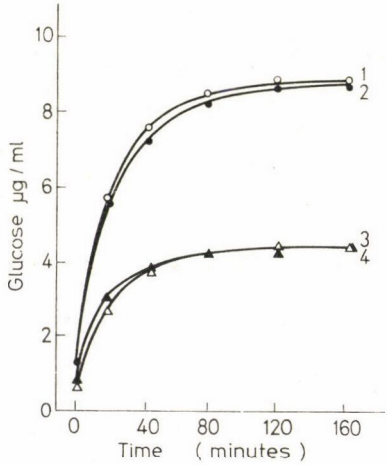

Fig. 8. Effect of sorbic acid on the sucrose- and maltose-decomposing activity of the cell-free extract of *Procandida albicans*. Incubation system: 1.6 ml cell-free extract in 1/15 M phosphate buffer (pH 5.5), 0.2 ml of 20% sucrose (1 and 2) or maltose (3 and 4), further 0.2 ml distilled water (control: 1 and 3), or 0.2 ml of 15 mg/ml SA solution (2 and 4), Incubation at 25 °C. Determination of glucose with glucose-oxidase reagent

cell-free extract of *C. claussenii* is demonstrated in Fig. 7, in the cell-free extract of *Pc. albicans* in Fig. 8, and in the acetone treated cells of *S. carlsbergensis* in Fig. 9. SA failed to inhibit enzyme activity in either case presented.

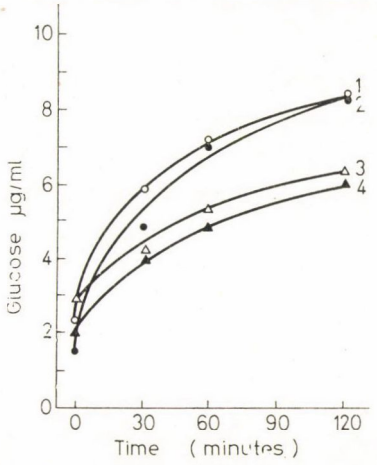

Fig. 9. Effect of sorbic acid on the sucrose- and maltose-decomposing activity of acetone of *Saccharomyces carlsbergensis*. Incubation system: 1.6 ml of acetone preparation in 1/15 M phosphate buffer (pH 5.5), 0.2 ml of 20% sucrose (1 and 2) or maltose (3 and 4), further 0.2 ml distilled water (control: 1 and 3) or 0.2 ml of 15 mg/ml SA solution (2 and 4). Incubation at 25 °C. Determination of glucose with glucose-oxidase reagent

3. Conclusions

The purpose of the examinations was to study the effect of SA on the carbohydrate fermenting activity of yeasts, in intact cells. In *in vivo* experiments it is rather difficult to interpret the effect of the inhibitor in terms of individual metabolic steps. However the value of information obtained from these experiments can be increased by studying not only one but several species which differ from each other in certain biochemical characteristics, and then evaluate the results by comparing them. *In vitro* studies on the effect of SA on different enzymes of the carbohydrate metabolism were performed only, when the inhibitory effect proved to be at least presumable in experiments with intact cells, by comparative evaluation.

It is known that, in the carbohydrate metabolism of the yeasts, the course of decomposition is identical with each substrate — included in these experiments — from the fructose-6-phosphate step on and the difference appearing in the carbohydrate utilization of certain species might be explained by the diversity of the initial steps of the carbohydrate metabolism (Sols & Fuente, 1961; Novák & Zsolt, 1962; Novák, 1966). These initial steps in case of the carbohydrates studies are presented in Fig. 10. Thus to evaluate the effect of SA from the viewpoint of carbohydrate metabolism, the differences in the

metabolism of the substrates studied are to be considered. In comparing the species and substrates, it seems to be the most effective method to start from the inhibition of the glucose fermentation. The cause of the difference of SA inhibition between the given carbohydrate and the glucose must be sought

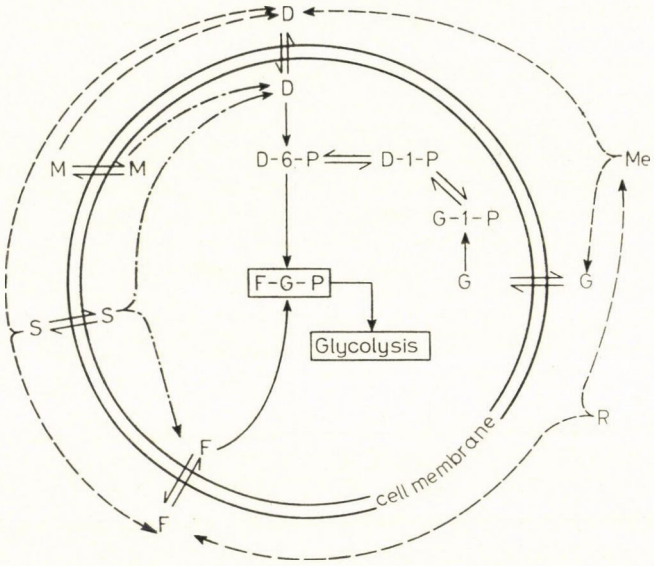

Fig. 10. Pattern of the initial steps of uptake and decomposition of the carbohydrates studied. See detailed description in the text. D: glucose, G: galactose, F: fructose, S: sucrose, M: maltose, R: raffinose, Me: melibiose, D-6-P: glucose-6-phosphate, G-1-P: galactose-1-phosphate, F-6-P: fructose-6-phosphate. — — —: extracellular splitting, —·—·—: intracellular splitting

namely in the diversity of the initial steps of their metabolism. The evaluation has been performed hereafter from this viewpoint, taking into consideration the effect of SA also on the oligosaccharide-splitting enzymes.

3.1. Effect of sorbic acid on maltose fermentation

The inhibition of SA proved to be stronger in the case of maltose fermentation than in that of the glucose with each of the species studied, except *C. claussenii* (Fig. 2 and Tables 1 and 2). It is known that *C. claussenii* decomposes the maltose with exomaltase (NOVÁK et al., 1966; TÜSKE, 1967), while every other species with endomaltase (PHILLIPS, 1959; NOVÁK, 1961; NOVÁK & ZSOLT, 1963a, b; NOVÁK et al., 1966; HARRIS & MILLIN, 1963). Thus, except for splitting with exomaltase, there is no difference in the metabolism of glucose and maltose in case of *C. claussenii*, as the transformation of maltose into glucose occurs outside the cell (Fig. 10). On the other hand, with the other endomaltase containing species the steps of uptake and intracellular splitting

of maltose differ from the metabolism of glucose (Fig. 10), thus the reason for the stronger SA inhibition might be found in these two steps. According to our experiments, however, SA failed to influence the activity of the maltose splitting enzymes (Figs 8 and 9), therefore it seems to be very likely that the effect of SA on maltose transport may cause the difference observed. Here we point out the fact, that as the degree of SA inhibition was determined in relation to the inhibition of glucose-fermentation, the glucose contamination of the maltose did not influence the evaluation of results.

3.2. Effect of sorbic acid on the fermentation of sucrose and raffinose

Of the species studied *C. utilis*, *S. carlsbergensis* and *S. cerevisiae* split sucrose and raffinose extracellularly (HESTRIN & LINDEGREEN, 1952; DWORSCHACK & WICKERHAM, 1961; NOVÁK, 1966; ZSOLT & NOVÁK, 1969), thus these substrates are transformed completely or partly into monosaccharides, transported by the glucose uptake system and phosphorylated by hexokinase (Fig. 10). This explains the fact, that the differences in the SA inhibition of the sucrose- and raffinose-fermentation were not significant as compared with that of glucose (Tables 1 and 2, Fig. 6). In case of *S. carlsbergensis*, however, contrarily to the manometric tests the Einhorn tests showed the inhibition of raffinose fermentation to be stronger than that of glucose or sucrose (Table 2). As the Einhorn tests needed a fermentation period of more than two days (Fig. 1) in contrast to the two hours required by the manometric experiments (Fig. 6) in the case of raffinose fermentation not only the first acting invertase had to be taken into consideration, but also the succeeding melibiase enzyme. This enzyme splits the melibiose remaining from the raffinose (Fig. 10). The strong inhibition observed might be caused in this case by the SA effect on the melibiase production or activity, or on the metabolism of galactose, derived from decomposition. The latter theory seems to be more probable, as galactose fermentation was more effectively inhibited by SA than that of glucose with *S. carlsbergensis* and the other species examined (Table 1).

In contrast to the three former species, endosaccharase functions in the sucrose metabolism of *Pc. tropicalis* (NOVÁK et al., 1966) and the inhibition of fermentation was stronger than that of the glucose (Fig. 5). For this phenomenon the effect of SA on the uptake and splitting of sucrose might be responsible, since just as with maltose these two steps differ from the metabolism of glucose (Fig. 10). SA, on the other hand, failed to inhibit endosaccharase (Figs 7 and 8) similarly to endomaltase, which refers again to the influence of SA on the transport activity.

3.3. Effect of sorbic acid on galactose fermentation

Several steps of the galactose metabolism differ from that of the glucose. These are the uptake and the four-step reaction of the waldenase system

(KALCKAR, 1958; Fig. 10). One of them might be more sensitive to the effect of SA, than the further steps of metabolism, which are identical with those of glucose. This can be the reason for the increased inhibition of the galactose fermentation (Table 1). A similar effect might occur also if in case of the inductivity of any of the above steps, SA inhibits the synthesis of the corresponding enzyme. The range of possibilities is too wide to draw conclusions from our experiments regarding the real cause of the difference found in the SA effect, in comparison with glucose. We point out, however, that galactose fermentation is in many cases, thus with *Pc. tropicalis* a labile and feeble characteristic (NOVÁK & ZSOLT, 1961, 1964), which might be ascribed to the inductive nature of galactose uptake or of the galactowaldenase system (ROBICHON-SZULMAJSTER, 1958). The inhibition of the energy requiring inductive processes by SA might occur readily and thus can provide explanation for the inhibition of fermentation in the degree observed.

3.4. Effect of sorbic acid on the uptake processes

Unequivocal results of fermentation experiments with maltose, sucrose and raffinose suggest that the SA inhibition of the fermentation of the intracellularly split oligosaccharides was more effective than that of the extracellularly split ones as compared to that of glucose. Since in the former case the uptake and splitting are the steps differing from that of the glucose, and as the activity of the splitting enzymes was not influenced by SA, it might be presumed that the cause of SA inhibition may lie with the uptake steps. Our previous experiments concerning the effect of SA on the carbohydrate assimilation of different yeasts lead to the same conclusion (DEÁK & NOVÁK, 1970).

Our direct experiments on SA affecting the uptake processes confirmed also these conclusions. These results show that the active transport of monosaccharides in the case of certain yeasts (KOTYK & HÖFER, 1965; DEÁK & KOTYK, 1968; DEÁK & NOVÁK, 1969a) is inhibited by SA, through uncoupling the energy supply necessary to this process (DEÁK & NOVÁK, 1969b). These experiments suggest that the inhibition of the active transport may be likewise responsible for the effect of SA on oligosaccharide fermentation, as according to the literary data the transport of yeast oligosaccharides is also an active uptake process (cf. NOVÁK & DEÁK, 1969).

Our experiments suggest that the effect of SA is demonstrable not only in the general and common pathways of metabolism (glycolysis, respiration), but manifests itself also specifically in the metabolic steps characteristic of the individual species, thus firstly in the carbohydrate transport. The inhibition of the active transport processes might be considered as the primary site of SA effect in the carbohydrate metabolism of yeasts. This effect is primary in the sense that it is the transport process by which the metabolism of the carbohydrates of external origin begins.

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CAROTENOID SYNTHESIS IN SEASONING PAPRIKA

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The red colour of the seasoning paprika derives from pigments, which consist of red and yellow carotenoids. The trade-value of red paprika is determined primarily by its colouring matter content, this being thus the most important measure of value. A method to determine the quantity of colouring matter is available.

The paprika harvested in red and ripe state, continues to live, its colouring matter content increases, while the sugar content decreases. Therefore the paprika must be after-ripened for 4 to 6 weeks before processing.

The carotenoid synthesis takes place in two phases. The synthesis in the first phase occurs in the fruit still on the plant and in the second in harvested fruits (after-ripening). The second phase reveals very intensive synthesis which exceeds the first phase by 150% in some cases, the chlorophyll disappearing completely by this time. While sunlight is required in the first phase, synthesis in the second phase is accomplished also in its absence. The β-carotine content of the paprika after-ripened in places without sunlight, shows an approx. 25% decrease. The carotenoid synthesis in the after-ripened paprika supports definitely the assumption, at least in relation to the oxygen containing carotenoids (xanthophyll), that chlorophyll has no direct role in the synthesis of carotenoids. The fruit of the seasoning paprika reveals very active respiration after harvest, which refers to intensive metabolic processes. According to studies on the respiratory quotient, organic acids are the basic materials of respiration. The decrease of the sugar content observed during the after-ripening period might be interpreted by the decomposition of the sugars into organic acids, a part of which gets utilized in respiration, and the other part serves as basic material for the carotenoid synthesis. The sucrose content has a significant role in this process.

The withering paprika left on the stock in red ripe state shows active vital functions, which result in a somewhat higher level of colouring matter than that observed in the paprika after-ripened for identical time following harvest. More favourable results are obtained by leaving red ripe paprika to wither on the stock for 3 weeks, and then after-ripening it for further 3 weeks after harvest.

Mature but still unripe paprika when harvested becomes red later on, however it contains less colouring matter than the paprika harvested red-ripe, both being subject to after-ripening.

The elongated pod of the seasoning paprika, Capsicum annuum L. var. longum, undergoes a very vivid change of colour before getting red-ripe. The dark green colour of the pericarp changes into blackish brown, then into spotted rusty brown and becomes finally red when ripe. The green colour of the pericarp, resulting from chlorophyll, decreases proportionally due to the formation of carotenoid colouring matter (pigments), the chlorophyll disappears finally and red becomes the ruling colour.

The red colour of the ripe fruit of the seasoning paprika comes from the yellow and red carotenoids, thus from plant colouring matters. The carotenoids

are polyenes with several conjugated double bonds and 40 carbon atoms, and belong to the group of compounds with isoprene skeleton. Because of the long hydrocarbon chain they dissolve in fat, thus being called also lipochrome. The carotenoids develop in plants and get into the animal organism with food.

The oxigen containing carotenoids are called xanthophylls or polyene-alcohols. According to CHOLNOKY (1937), the polyene-alcohols are present in esterized form in the paprika, bound to fatty acids of long carbon chains. The "paprika red" is thus a pigment-wax. The esterification occurs with ripening. The scarlet colour of paprika is caused mainly by the capsanthin ($C_{40}H_{58}O_3$) and the capsorubin ($C_{40}H_{60}O_4$), the other yellow colouring matters have only subordinate role in the formation of colour. The red pigments mentioned dominate also quantitatively, their intensity being about ten times higher than that of the yellow alcohols and carotenes. The β-carotene ($C_{40}H_{56}$) and the kryptoxanthin ($C_{40}H_{56}O$) of the yellow pigment turn into vitamin A in the organism, the yellow zeaxanthin ($C_{40}H_{56}O_2$) is also important. Only traces of α-carotene can be found in the paprika. The great number of colouring matters of the paprika occur only in small quantities in the pericarp, and their crystallization proved to be very difficult. It is due to the work of KARRER (1948), that 20 kinds of pigments are known at present. This author determined the structure of the carotenoid-epoxides, and elaborated a technique to identify the pigments without crystallization.

Ground paprika is produced generally by grinding in different proportions the dry pericarp together with the paprika seed of high oil content. Hungarian red paprika is provided with quality mark and lead seal when placed on the market. According to our experience the colour of the ground paprika derived from paprika pods harvested at the beginning of the ripening season and processed immediately is light-red, tastes sweeter than usual, while those processed later showed a deeper red colour and were less sweet.

These findings obtained by sensory evaluation brought us to study the cause of this phenomenon. We considered it important because the right colour of ground paprika is an essential quality requirement. The darker and more flaming red it is, the higher grade and price can be obtained for the product.

The deepening of the colour of the paprika harvested red ripe was ascribed merely to drying. Our observation suggests that drying is accompanied by the subsequent formation of colouring matter, i.e. the pigment content increases as related to the dry matter. This process has been called after-ripening. Recognizing the importance of the problem, the paprika-processing industry considered necessary to determine the time when the pigment formation following the harvest has been completed and processing can be started without loss of colouring matter. Out of the accompanying phenomena, the changes of the sugar content were found to be of greatest importance, and problems

arising in plant physiology justified likewise the extensive study of this question.

We have published several papers on the topic of after-ripening of the paprika. Our present publication aims to give a short and comprehensive report on this problem, to draw new conclusions and to get better knowledge on the carotenoid synthesis.

1. Materials and methods

Parallel experiments were performed with pungent and sweet seasoning paprika varieties. The pungent paprika products of excellent quality are processed from regional paprika varieties of Szeged. Sweet varieties were grown by HORVÁTH (1935) in Kalocsa, by selecting the mildly hot or sweet fruits when evaluating paprika. The sweet seasoning paprika which developed presumably from random hybridization between sweet paprika and pungent seasoning paprika, differed from the latter not only in the lack of capsaicin, the pungent agent in paprika, but revealed also a lower inner value. However the processing of the sweet paprika variety is easier and cheaper than that of the pungent one, and this fact became in the course of time a decisive factor. Its drawbacks, thus the lower colouring matter and high sugar content were recently successfully eliminated by plant breeders. The unfavourable effect of the sugar content manifests itself during the drying and grinding procedures of the pericarp.

The higher the sugar content of the pericarp the more pronounced the colour and flavour deficiencies appear, these being due to caramelization and to the formation of reductons, melanoids etc.

The differences between the two paprika varieties are now less apparent, the sweet paprika having joined up, as a result of plant improvement activities, the hot type, its sugar content became lower and the pigment content higher.

Experimental data on which our first conclusions are based (BENEDEK, 1952) were obtained nearly 20 years ago, and a part of the data presented in this paper (Tables 1—4) are results of experiments carried out nearly 15 years ago (BENEDEK, 1959). They cannot be reproduced anymore and are very important because the differences between the composition of the two paprika varieties which provided the basis for drawing the conclusions, have almost disappeared since.

The determination of the colouring matter content was carried out by a widely used simple and rapid method (BENEDEK, 1958) which is performed by extracting all the pigment from the ground paprika with benzene and determining the optical density of the solution in a spectrophotometer at 492 nm wavelength. The colouring matter content is expressed in capsanthin, the most significant paprika pigment. The pigment content expressed in g/kg capsanthin

characterizes with accuracy the colouring capacity. The colouring matter content means further, all the pigments of paprika, as determined by the above method.

The quantity of the individual pigments has been determined with the absorption chromatography technique of CHOLNOKY (1937).

The sugar content was determined by iodometry. The total sugar content represents the amount of reducing sugar and sucrose, as calculated in glucose.

Experiments were carried out with the fruits of improved seasoning paprika strains, harvested for the first time, in red ripe intact state, at the end of August or early September. The pericarps of a small quantity (about 20 pieces) of fruit were dried directly after harvest and prepared to be examined, while the greater part was stringed and stored hanging on the conventional racks in a sunny place. The most valuable part of the seasoning paprika is the pericarp, which contains the seasoning materials, thus our studies were focussed on this part of the plant. Depending on their moisture content the pericarps were dried for several hours at 67 °C (± 2), this drying procedure was repeated for further 2 to 3 hours after grinding. Gentle drying is indicated because the carotenoids break up easily while double drying is needed because of the moisture absorbing capacity of the pericarp. Samples were taken and the examinations were repeated weekly. The results from experiments performed with pungent and sweet paprika varieties for several years, are demonstrated in Tables and diagrams.

2. Results

2.1. After-ripening in sunlight

The carotenoid synthesis and the accompanying sugar decomposition represent the most significant procedures of after-ripening in the pericarp of the seasoning paprika. The following Tables and Figures present the results of experiments concerning this problem. The Table shows a 120% increase of colouring matter content found in the pungent seasoning paprika. This increase was unexpectedly high. Five to 6 weeks after harvest the increase stops. The increase of colouring matter content in the sweet seasoning paprika is only about 70% and the synthesis ceases earlier, 3 to 4 weeks after harvest. The graphic analysis of the two experiments shows the pigment increase to be more powerful and the after-ripening more favourable in the case of pungent paprika. In certain cases the total sugar content and pigment content of the fruits were determined simultaneously. Changes observed in the total sugar content of pungent paprika are summarized in Table 3, while those obtained with the sweet variety are demonstrated in Table 4. Changes of the mean values in Tables 3 and 4 are shown in Fig. 2. The above results show that the pungent paprika loses during the after-ripening period in the average 32% and the

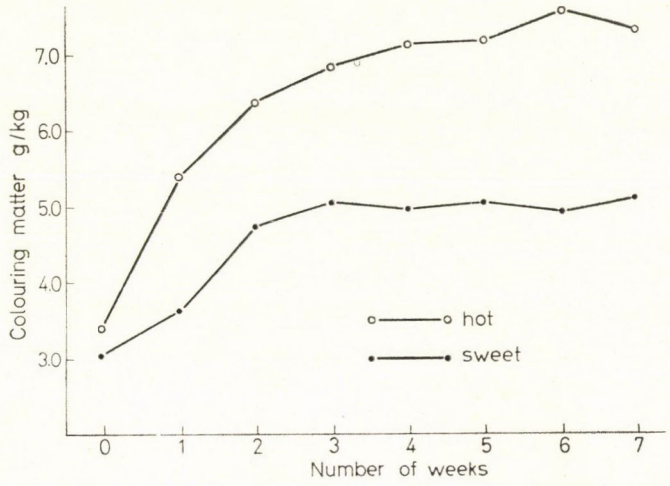

Fig. 1. Increase of colouring matter content in hot and sweet seasoning paprika kept in sunlight

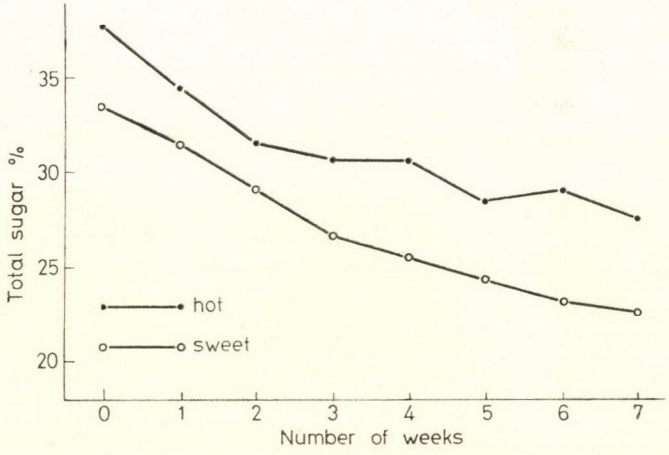

Fig. 2. Decrease of the total sugar content in hot and sweet seasoning paprika kept in sunlight

sweet variety 25% of its total sugar content as determined directly after harvest. On the other hand the colouring matter content of the hot paprika shows 120% and that of the sweet variety 70% increase as compared with the quantities determined upon harvest.

Data summarized in Table 5 offer a more detailed study of the changes accompanying carotenoid synthesis. The experiment gives informations on the changes of xanthophyll, β-carotene, sucrose, reducing sugar and total sugar content in the growth and ripening phase as well as in the paprika pods

Table 1

Increase of colouring matter content in hot paprika kept outdoors in sunlight

Serial number	Colouring matter in dry matter, g/kg							
	at harvest	after harvest						
		1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks
1.	3.40	6.40	6.90	7.40	7.30	7.50	8.20	7.90
2.	3.88	5.99	6.44	7.48	7.37	8.17	7.96	8.65
3.	2.93	4.91	6.07	5.77	5.97	6.62	6.64	7.09
4.	3.97	4.41	6.32	6.07	6.52	7.24	6.84	6.87
5.	3.19	4.81	5.37	5.57	6.62	6.07	6.52	6.82
6.	3.24	5.42	6.22	6.94	7.62	7.57	7.76	7.34
7.	3.37	6.09	6.72	7.37	7.72	8.64	8.17	7.39
8.	3.09	5.98	6.89	7.12	8.37	8.12	7.74	7.44
9.	3.80	5.75	7.55	7.86	7.79	7.43	8.27	7.29
10.	3.47	4.71	5.70	6.29	6.77	6.42	7.19	6.99
mean value	3.43	5.44	6.41	6.78	7.20	7.37	7.52	7.37
$S\,\bar{X}$	$\pm$0.0357	$\pm$0.0656	$\pm$0.0633	$\pm$0.0799	$\pm$0.0915	$\pm$0.0819	$\pm$0.0671	$\pm$0.0591
increase per cent	—	58.60	86.88	97.66	109.91	114.60	119.24	114.86

$S\,\bar{X}$ deviation of mean values

Table 2

Increase of colouring matter content in sweet paprika kept outdoors in sunlight

Serial number	Colouring matter in dry matter, g/kg							
	at harvest	after harvest						
		1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks
1.	3.50	4.30	5.20	4.90	5.20	4.90	5.20	5.70
2.	3.30	4.40	5.76	6.78	5.78	6.65	5.45	6.26
3.	3.47	3.57	5.01	5.67	5.01	5.17	5.08	5.67
4.	2.93	3.07	4.71	4.41	4.81	5.37	5.08	5.01
5.	2.93	3.27	4.41	5.17	4.91	5.01	4.83	4.61
6.	1.85	3.46	4.06	4.02	4.34	4.28	4.49	4.00
7.	3.32	3.62	4.11	4.25	4.36	4.43	4.38	4.56
mean value	3.04	3.67	4.75	5.02	4.91	5.11	4.93	5.11
$S\,\bar{X}$	$\pm$0.217	$\pm$0.189	$\pm$0.233	$\pm$0.362	$\pm$0.182	$\pm$0.294	$\pm$0.145	$\pm$0.299
increase per cent		20.72	56.25	65.13	61.51	68.09	62.17	68.09

$S\,\bar{X}$ deviation of mean values

Table 3

Decrease of total sugar content in hot paprika kept outdoors in sunlight

Serial number	Total sugar in dry matter, per cent							
	at harvest	after harvest						
		1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks
1.	32.35	28.02	28.40	25.09	20.05	19.24	19.09	16.94
2.	37.83	35.12	32.28	28.60	31.74	26.94	28.50	24.17
3.	36.18	34.39	31.67	28.88	29.36	27.04	27.75	25.75
4.	38.85	38.14	34.49	31.00	32.60	30.79	28.76	28.09
5.	27.09	27.58	25.45	25.42	23.02	22.15	19.28	21.11
6.	32.52	30.74	30.19	24.50	23.95	20.08	20.39	21.85
7.	33.30	30.11	24.31	23.28	18.13	24.41	19.56	21.05
8.	29.81	28.47	27.18	28.02	25.11	24.23	23.47	23.15
mean value	34.49	31.57	29.24	26.84	25.49	24.37	23.35	22.76
S $\overline{X}$	1.46	±1.49	±1.24	±0.93	±1.87	±1.35	±1.54	±1.19
increase per cent		5.73	12.69	19.85	23.88	27.23	30.27	32.03

S $\overline{X}$ deviation of mean values.

Table 4

Decrease of total sugar content in sweet paprika kept outdoors in sunlight

Serial number	Total sugar in dry matter, per cent							
	at harvest	after harvest						
		1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks
1.	38.50	35.71	30.86	32.35	33.61	30.45	29.41	30.26
2.	39.74	35.97	32.97	28.94	30.79	30.01	29.37	26.98
3.	39.10	35.42	31.44	29.12	28.59	25.06	26.77	25.83
4.	40.29	32.78	31.10	31.78	29.02	27.43	28.91	27.92
5.	31.51	32.12	31.40	31.55	32.14	29.28	30.61	27.78
mean value	37.82	34.40	31.55	30.74	30.83	28.44	29.01	27.75
S $\overline{X}$	1.61	±0.80	±0.36	±0.71	±0.94	±0.98	±0.62	±0.71
increase per cent		9.04	16.57	18.72	18.48	24.80	23.29	26.62

S $\overline{X}$ deviation of mean values.

Table 5

Changes occurring in growing, ripening, red ripe harvested and after-ripened fruits. The first sample was taken (0 week) of 5 cm long fruits. Data refer to dry matter

Time in weeks	0	2	4	5	6*	8	10	12
				hot				
Xanthophylls g/kg	—	—	0.69	3.80	4.41	7.76	7.88	8.65
β-carotene g/kg	0.03	0.04	0.07	0.14	0.37	0.42	0.49	0.52
Sucrose %	6.13	6.45	12.53	5.37	3.27	2.11	1.37	0.76
Reducing sugar % (as glucose)	11.81	12.18	15.38	22.84	22.91	21.92	20.64	19.84
Total sugar %	17.94	18.63	27.91	28.21	26.18	24.03	22.01	20.60
				sweet				
Xanthophylls g/kg			0.66	2.65	3.47	5.95	6.44	6.82
β-carotene g/kg	0.04	0.04	0.06	0.13	0.25	0.32	0.35	0.35
Sucrose %	7.06	6.69	11.08	6.20	2.25	2.80	1.74	1.43
Reducing sugar % (as glucose)	9.37	12.04	19.84	25.22	32.30	23.60	23.20	22.17
Total sugar %	16.43	18.73	30.92	31.42	34.55	26.40	24.94	23.60

* end of the red ripening phase in the sixth week

harvested red ripe and after-ripened. No measurable quantity of pigment can be found in the green paprika during the first weeks, it contains only a small quantity of β-carotene. About 5 cm long unripe green fruits were examined at first. The ripening phase i.e. the colouring began during the fourth week and was finished with the red ripening during the sixth week (marked 6*). As the most interesting changes occur in the ripening phase, data of the intervening fifth week are also presented. Experiments performed repeatedly for several years with different varieties showed essentially identical results. Conclusions remained the same, only the value-proportion of the analytical data revealed slight deviations. The sweet paprika variety was characterized in these experiments also by higher sugar content and lower pigment content. Experiments described here were performed in 1963.

2.2. After-ripening in the dark

Experiments were carried out with paprika fruits harvested in red ripe state, subsequently stringed and stored in dark but spacious and airy places. The strings for comparative studies were kept on racks in sunlight. Beside the pigment content provitamin A i.e. β-carotene and kryptoxanthin were

Table 6

After-ripening experiments with hot paprika kept in sunlight and in the dark

Serial number	State	Kept in sunlight			Kept in the dark		
		colouring matter	β-carotene	krypto-xanthine	colouring matter	β-carotene	krypto-xanthine
		g/kg in dry matter					
1.	At harvest	4.71	0.26	0.09	4.19	0.25	0.09
2.	after 1 week	6.06*	0.46*	0.16*	5.84	0.38	0.15
3.	after 2 weeks	7.42	0.49*	0.20*	7.94*	0.47	0.16
4.	after 3 weeks	8.53*	0.50*	0.21*	8.08	0.47	0.18
5.	after 4 weeks	8.70	0.61*	0.21*	9.94*	0.56	0.17
6.	after 5 weeks	10.48	0.69*	0.23	10.91*	0.47	0.24*
7.	after 6 weeks	10.15*	0.69*	0.27*	9.20	0.41	0.21
8.	after 7 weeks	9.58	0.57*	0.24*	11.06*	0.45	0.23
9.	after 8 weeks	9.75	0.46	0.26*	11.72*	0.50	0.24
10.	after 9 weeks	10.16	0.64*	0.28*	11.70*	0.45	0.26

* higher value

Table 7

After-ripening experiments with sweet paprika kept in sunlight and in the dark

Serial number	State	Kept in sunlight			Kept in the dark		
		colouring matter	β-carotene	krypto-xanthine	colouring matter	β-carotene	krypto-xanthine
		g/kg in dry matter					
1.	At harvest	2.23	0.13	0.05	2.45	0.13	0.05
2.	after 1 week	3.39	0.17	0.07	3.42*	0.17	0.08*
3.	after 2 weeks	4.79*	0.21*	0.09*	4.66	0.19	0.08
4.	after 3 weeks	4.73	0.28*	0.08	4.76*	0.20	0.09*
5.	after 4 weeks	4.98	0.24*	0.09*	5.11*	0.20	0.08
6.	after 5 weeks	5.33	0.30*	0.10	5.97*	0.25	0.12*
7.	after 6 weeks	4.83	0.31*	0.13*	5.37*	0.30	0.12
8.	after 7 weeks	5.26	0.18*	0.14*	5.43*	0.17	0.12
9.	aftre 8 weeks	5.46	0.25*	0.13*	5.89*	0.17	0.11
10.	after 9 weeks	5.57*	0.30*	0.10	5.55	0.19	0.12*

* higher value

determined. Examinations were performed with both pungent and sweet paprika varieties.

Data of the tables show that paprika pods when kept in sunshine contain more carotene and the storage in the dark influences unfavourably the synthesis of provitamin A, which is important from the viewpoint of nutritional biology. This effect can be observed in a lesser degree in the case of kryptoxanthin which exerts also provitamin A effect.

After-ripening in the dark influences somewhat more favourably the synthesis of the colouring matter content than the after-ripening in sunlight.

2.3. Double after-ripening

Experiments were performed with pungent (serial number 1—4) and sweet (serial number 5—7) varieties by harvesting a part of the red ripe fruits. They were stringed and kept in sunlight for 6 weeks. The other part of the red ripe fruits was marked and subjected to after-ripening for three weeks on the plant and for three more weeks after being harvested. The latter system was called double after-ripening. Experimental data on the paprika left red ripe on the stock for three weeks are presented separately. Examinations included both the colouring matter and the total sugar content.

The favourable effect of the double after-ripening method manifests itself in the higher colouring matter and lower sugar content obtained.

Table 8

Changes of colouring matter and sugar content in the paprika after-ripened for 6 weeks following harvest, for 3 weeks on the plant, and for 3 weeks on the plant and further 3 weeks after harvest

Serial number	Stock	At harvest		After-ripened for 6 weeks following harvest		After-ripened for 3 weeks on the plant		Double after-ripening, 3 weeks on the plant 3 weeks after harvest	
		colouring matter g/kg	total sugar %	colouring matter g/kg	total sugar %	colouring matter g/kg	total sugar %	colouring matter g/kg	total sugar %
1.	163	3.79	33.38	6.82	23.24	6.32	26.13	7.77	19.81
2.	K-69	3.54	27.95	6.82	22.12	7.19	24.62	8.04	20.90
3.	K-73	3.89	25.23	7.82	21.63	6.82	23.60	8.17	18.71
4.	K-7	3.39	30.24	7.74	22.56	5.30	24.82	8.94	16.48
5.	57—13	3.12	34.06	7.09	23.48	6.63	27.69	7.95	22.07
6.	54—6	2.43	34.90	6.12	27.88	5.22	28.26	6.14	25.69
7.	82 543	2.83	35.46	5.26	26.74	5.65	30.62	5.97	26.23
	mean value	3.28	31.31	6.81	23.95	6.16	26.53	7.85	21.42
	$S\,\bar{X}$	±0.198	±0.474	±0.339	±0.907	±0.293	±0.856	±0.424	±1.379

$S\,\bar{X}$ deviation of mean values

3. Conclusions

3.1. After-ripening in sunlight

Data presented are, despite of using average samples, not always perfectly consequent, several outstanding values can be found among them. This phenomenon is met also when other plant materials are studied and can be explained by the inhomogeneity of the material examined. Studying the fruits of improved seasoning paprika, this being from morphological viewpoint the most stable type, significant deviations were found in the quality characteristics not only of fruits derived from different stocks, but also those grown on the same plant. The quality characteristics are influenced by the weather and soil conditions as well as by other factors. Data of Tables 1—4 demonstrate that the total sugar content which is in the average lower in the pungent variety than in the sweet one, decreases gradually during after-ripening, while the colouring matter content increases. The strong decrease of the sugar content in hot paprika is followed by a strong pigment increase, while both processes are insignificant in the sweet type. The relation between the colouring matter (carotenoid) synthesis and the total sugar content suggests that not only respiration, but also the carotenoid synthesis reduces the sugar content of the pericarp. Had the sugar deficiency been used only for respiratory purposes, its decrease would have been identical in both paprika varieties, during the carotenoid synthesis.

According to data presented in Table 5 β-carotene can be demonstrated as early as in the developing fruit. This is the only paprika carotenoid which synthesizes without the visible decomposition of chlorophyll, while the synthesis of the other oxygen containing carotenoids, i.e. xantophylls, is accompanied by the decomposition of chlorophyll, and this is manifested in the change of colour of the pericarp.

The sucrose content decreases considerably in the ripening phase when the synthesis of the xantophyll begins, while the reducing sugar content keeps increasing and starts to decrease only later in the red ripening phase, or even after it. This decrease is, however, essentially not as extensive as that of the sucrose content. The approx. 90% loss of the sucrose content refers to its significant role in the carotenoid synthesis.

The carotenoids are compounds of isoprene skeleton, and contain 40 carbon atoms in accordance with the 8 isoprene skeletons. BONNER (1950) supposed that the synthesis started from a base compound with ramified chains, possessing 5 carbon atoms. The nature of this base compound has not been defined so far, it is supposed that an organic acid of 5 carbon atoms has a role in it. Since, as supposed, the direction of polimerization turns in the middle of the chain in all the carotenoids, it might be presumed that polymerization continues as far as an intermediate product C_{20} (or products), and

the final chain is formed by the connection of two such chains. According to WILLSTÄTTER & MIEG (1907), an alcohol, the phytol which is a component of chlorophyll, might be regarded as the base compound of carotenoid synthesis. This is namely the only known C_{20} base compound occurring in plants, and in the opinion of these authors the carotenoid synthesis is, indeed, active in plants which develop large amounts of phytol.

However, carotenoid synthesis takes place also in organs deficient in chlorophyll, such as beets, a number of seeds and flowers, and this fact contradicts the former theory. It occurs also in fruits which are green when unripe, such as tomato, where the quantity of phytol released following the disappearance of chlorophyll is relatively small as compared to the amount of lycopin formed. Thus one may presume that, though the phytol acts in carotenoid synthesis as starting compound, its formation is not necessarily connected with chlorophyll synthesis. It is most probable that phytol is rather a reduction product of the base compound common to chlorophyll and carotenoids, than the base compound itself (BONNER, 1950).

The findings obtained with tomato apply also to paprika, carotenoid synthesis being, indeed, active only when the chlorophyll has disappeared completely. This phenomenon contradicts sharply the theory of WILLSTÄTTER and MIEG. However there is a phase of synthesis in the growing plant which is in accordance with their theory. The β-carotene synthesis occurs namely — as indicated earlier — without visible decomposition of the chlorophyll, in the green unripe paprika. The chlorophyll begins to decompose only later, in the phase of ripening, when the synthesis of the oxygen containing carotenoids (xantophylls) starts. Thus, it might be supposed that the synthesis of β-carotene and the xantophylls occurs under different conditions.

The following 3 phases can be distinguished during carotenoid synthesis, considering also the changes of sugar content:

a) The synthesis of β-carotene takes place in the presence of chlorophyll, accompanied by the increase of the sugar content.

b) The β-carotene synthesis is followed by the synthesis of xantophylls in the ripening phase (4 to 6 weeks), with simultaneous significant decrease of the sucrose content.

c) The intensive xantophyll synthesis in the paprika harvested red ripe is followed by the decrease of the reducing sugar content.

GRACZA and BENEDEK (1961) performed experiments to elucidate the problems of plant physiology arisen. Respiration is intense at the beginning in the harvested withering fruit, later it decreases gradually and ceases finally within 30 to 40 days, as measured by GRACZA. This fact shows metabolic activity in the ripe fruit separated from the plant. GRACZA found the respiratory quotient (RQ) to be higher than 1. This suggests that the basic materials of respiration are not sugars but compounds of higher oxydation degree, such as

organic acids, of the plant. Probably a part of the sugars turns into organic acids by oxidation and this might be the explanation for the decrease of the sugar content. The organic acids serve partly for respiration, partly as the basic material of carotenoid synthesis. Thus the carbon skeleton of the acids derived from the oxydative decomposition of sugars might provide the basis of carotenoid synthesis. This hypothesis corresponds on the whole to the results of the experiments described above. Some practical relations of the problem are discussed here-after.

Our after-ripening experiments contradict the earlier conception that the deepening of the colour of the pericarp is a consequence of desiccation only. Though the life-conditions of the harvested paprika pods change essentially, the fruit carries on biological functioning and produces carotenoid pigment. If the fruits are dried directly or shortly after harvest, they wither because of the lack of water necessary to the functioning of the protoplasm, and the valuable colouring matter fails to form. Thus the time of processing influences the quality of ground paprika. The paprika processed without appropriate after-ripening gives a less valuable product of pale colour and low pigment content. The pungent paprika pod requires 5 to 6 weeks and the sweet variety 3 to 4 weeks of after-ripening before being processed.

To obtain standard data suitable for comparison, chemical analysis of the seasoning paprika has to be performed under the balanced conditions, following after-ripening.

It often occurs that a part of the harvested paprika pods is not perfectly ripe. Thus it seemed to be of interest to study the components of the after-ripened fruits gathered in ripe and unripe state. The processing industry lowers the value of a batch of goods containing unripe fruits too. In our experiments certain chemical components harvested green, in the phase of ripening and in red ripe state (BENEDEK, 1949) were analysed. The ether extract containing mainly fatty oils, lipids and waxes, increases significantly till the ripening phase, this increase slows down in the ripening phase till the red ripe state is reached.

The sand-free ash content of the pericarp (mineral substance) decreases gradually toward the ripening phase, and this is in connection with the migration of the mineral substances occurring during this period in the plant.

According to the examinations on the colouring matter content, the fruit must not be harvested before getting red ripe because the loss of colouring matter is felt even subsequent to after-ripening. Although the paprika harvested green becomes red ripe too, its colouring matter content will be low. Harvesting in the ripening phase caused a loss of 15% in the colouring matter content in our experiments.

The total sugar content increases significantly till the ripening phase, then to a lesser extent till the middle of this phase, hereafter it decreases and

during after-ripening, as shown already, the decrease is significant. The total sugar content is higher in fruits harvested unripe than in the ripe ones. As the high sugar content is unfavourable for the processing industry, early harvest is disadvantageous for both the colouring matter and the sugar content. Thus the paprika harvested before being red ripe, produces less valuable goods. Furthermore the weight of a 1 m^2 pericarp increases in the ripening phase, and as the weight is nearly proportional to the thickness of the pericarp, the pericarp of the paprika harvested unripe is thinner than that harvested ripe.

3.2. After-ripening in the dark

So far our findings in after-ripening experiments related to paprika pods stringed and kept on racks in sunlight. Experiments were performed to determine the effect of the lack of sunlight on the carotenoid synthesis. A part of the material used for outdoor examinations was stored in a shaded place. The colouring matter content of these pods was slightly higher than that of those kept in sunlight. If the after-ripening procedure aims to increase the colouring matter content, it seems to be important to know all the factors which improve quality.

Paprika fruits gathered when red ripe, stringed and stored in a dark but well aired place were used in the experiments. Control strings were kept in sunlight.

Experiments performed with pungent paprika variety (Table 6) revealed that after-ripening in the dark influenced the formation of colouring matter more favourably than after-ripening in sunlight. Leaving out of consideration the data of serial number 1 (values obtained at harvest), 6 out of 9 data support the former observation (values marked with *), which implies on the average 12.7 % increase of colouring matter content. Seven against two data confirm the advantage of after-ripening in the dark, in the case of the sweet variety (Table 7), showing an increase of 5.5 %, on the average.

In the case of β-carotene and kryptoxanthin, better results were obtained with after-ripening in sunlight than in the dark, the latter having caused a loss of about 25 %. The increase of colouring matter content being very slight, our experiments suggest merely that the after-ripening is accomplished also when the paprika pods are kept in shady or even dark places. The practical importance of this finding is, that the storage of the strings can be started directly after harvest under optimal indoor conditions.

Considering however the provitamin A content, storage in the dark caused in all the three experiments about 25 % loss in the β-carotene content, which fact can not be neglected form the viewpoint of nutritional biology. However, this does not involve the loss of commercial value, since the trade value of paprika is solely judged by its colouring matter content.

If the carotenoid synthesis is regarded as a two-phase process, it can be pointed out that, while it requires definitely sunlight in the first phase till red ripening is reached, subsequently in the after-ripening (second) phase it gets accomplished without sunlight, in the dark.

3.3. Double after-ripening

Experiments were carried out to compare the colouring matter and sugar content of red paprika fruits left on the plant, with that of fruit after-ripened for 6 weeks following harvest. The six-week period was divided in two parts, for this purpose, by after-ripening the fruits for 3 weeks on the plant and for further 3 weeks in harvested state, considering the fact that late harvest improves the quality (BENEDEK et al. 1964).

Data of the comparative studies presented in Table 8 show that the colouring matter content of the paprika pods after-ripened on the stock for 3 weeks is, on the average, lower than that of the pods after-ripened for six weeks following harvest. However, better colouring matter results were obtained with pods after-ripened for three weeks on the stock and for further three weeks after harvest than with those after-ripened for six weeks following harvest.

The better results thus obtained might be explained by the fact that though the paprika harvested red ripe continues to live, its synthesizing activity is lower than that of the fruits left on the stock, suggesting that red ripening does not involve the interruption of connection with the parent plant.

It is interesting to observe the development of the total sugar content during after-ripening which is in this case also inversely proportional to the colouring matter content. These experiments confirm also our theory that the carotenoid synthesis occurs at the charge of the sugar content.

These results suggest that a delayed harvest provides means to improve the colouring matter content. The practical application of double after-ripening will be greatly advanced by growing paprika simultaneously ripening and by the mechanization of harvesting.

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REDUCTION OF SPOILAGE LOSSES OF STRAWBERRIES BY IONIZING RADIATION

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Two series of experiments were carried out with the strawberry variety Senga Sengana. In the first series the fruit was irradiated in units of 0.5 kg and in the second in 0.2 kg units and stored at 12—14 °C and 22—24 °C temperature, respectively. A ^{60}Co panoramic gamma radiation source of 50 kCi activity was used at a dose rate of about 200 krad/hour.

The results of the experiments show that the loss due to spoilage during the 3-to 7-day periods between harvesting and marketing was reduced by treatment with 200—300 krad gamma radiation to one half, one fourth of the loss in the untreated control sample. It is not advisable to use radiation doses above 300 krad because of the danger of softening of the fruit and of reduced resistance to microorganisms. A decrease in the sensory quality as established by triangular test, was not observed even in strawberry samples treated with 400 krad.

The packaging materials and methods as applied at present in the storage and transport of strawberries can be used in case of radurization as well.

The strawberry is one of the most valuable fruits in Hungary with its high sensory value, early maturation time and export trade possibilities. During the last decade the amount grown in Hungary reached 10 000 tons as compared to the 1—2000 tons produced in the early 50-'s (ANON, 1965a, 1970). In 1969 for instance the total yield was about 10 600 tons (ANON, 1970). The value of the total annual yield, counting with an average price of 15 Ft/kg in the Hungarian market, is estimated at approximately 150 000 000 Ft. About 10 to 25% of the total yield used to be exported.

The export volume of strawberry and other berry fruit is limited only by the amount grown and the low keeping quality. The strawberry is one of the most perishable fruits. According to American literature (DROGE, 1965; ANON, 1965b; KETCHUM et al., 1965) about 13—25% of the crop is lost due to spoilage, during the period between harvesting and marketing. Estimating the spoilage in Hungary at about 10—15%, this means a loss of at least 15—20 million forints to national economy. Increasing the keeping quality even with a few days may thus have a great economic effect, not only by reducing the losses during the period prior to marketing, but also by the possibility of a substantial reduction of the transport costs. The extended storage life would permit of refrigerated transport instead of air transport, or extend the area of transportation, and thereby the export outlet. These considerations explain the interest shown abroad in studying the possibilities of increasing the keeping quality

of strawberries by radurization (England: HANNAN, 1955; CLARKE, 1959; USA: NELSON et al., 1959; MAXIE et al., 1964; JOHNSON et al., 1965; LOVELL & FLICK, 1966; SOMMER et al., 1968, and others; USSR: KOVALSKAYA et al., 1961; KUDRYASHOVA et al., 1967; France: VIDAL, 1966; Spain: FERNANDEZ Y GONZALEZ, 1967; GARCIA DE MATEOS et al., 1967; Israel: BARKALAI-GOLAN et al., 1967; Italy: BUDINI et al., 1968, etc.). The same considerations led our Institute as early as between the years 1962—64 (FARKAS & KISS, 1967) to carry out preliminary investigations with 7 strawberry varieties. Our work was followed by experiments carried out in cooperation with Soviet researchers (KISS et al., 1967).

As with other methods of preservation, the efficiency of radiation treatment is affected not only by storage conditions, but by the variety, maturity, physiological state, even by the growth conditions of the fruit in question. It is necessary, therefore, to control also in Hungary the reproducibility of results obtained abroad and to clarify those which can be applied under the local conditions.

1. Materials and methods

1.1. Test samples and their preparation

Two series of experiments were carried out. In both cases the strawberry variety Senga Sengana was used as test material. In the earlier experiments (FARKAS & KISS, 1967) this variety proved to be suitable for radiation treatment, the quantity of this variety of strawberry grown in Hungary is increasing from year to year and this is one of the varieties most suitable for canning as well (MOHÁCSY et al., 1965; GUTSCHMIDT, 1969).

The test material was purchased in both cases at the Szentendre Cold-Stores, Cooperative Marketing Centre of the County of Pest, on 17 June 1970 and 1 July 1970, respectively. The strawberry was bought and irradiated one day after the harvesting. Between harvesting and purchasing the fruit was stored in the Cold Stores at Szentendre at 5—10 °C. Refrigeration was not provided during transport.

On the first occasion, the test material consisted of sound, ripe fruit. In the second case, the fruit was over-ripe and neither the texture nor the microbiological condition were satisfactory.

In the first experimental series the whole test material has been treated without selection. In the second case the bruised or moulded fruits were removed. For the treatment the fruits were placed on the strawberry-trays of the Hungarofruct Co. (MOHÁCSY et al., 1965). The amounts per tray in the first and in the second experimental series were 0.5 kg and 0.2 kg, resp.

In the first experimental series the fruit was tested for the frequency distribution of weight in two trays selected by random sampling. In one of the

trays an average weight of 12.1 g was found for 25 pieces of fruit and ± 2.5 g standard deviation, in the other 12.8 g average weight with a standard deviation of ± 2.3 g. Thus the distribution according to weight in the trays could be considered sufficiently uniform. An average pH = 3.8 was observed in the juice pressed from 25 pieces, with ± 0.17 standard deviation. A similar distribution test showed the average refractive index of the soluble solids content to be $7.2 \pm 1.1\%$. No correlation was found between the weight of the single fruits and their pH and refractive index. So it did not seem necessary to sort the fruits according to their size for the later experiments.

1.2. Method of radiation treatment

The radiation treatment was carried out in a ^{60}Co panoramic gamma source of 50 kCi activity. The temperature in the radiation chamber was 14 °C. The dose rate in the place of exposure was established by chemical dosimetry. Ampoules containing Fricke's ferrous sulfate reagent were placed on the sample trays and exposed to approximately 200 krad/hour radiation treatment. The dosimeter ampoules were removed after 3 minutes treatment. The treatment of the fruits was continued for a predetermined period. The dosimeter solutions were evaluated by spectrophotometry and in the knowledge of the time of treatment the radiation dose absorbed by each sample could be calculated. This was necessary because of the inhomogeneity of dose distribution in the treatment chamber. In the first experimental series the samples stored later at a temperature of 12—14 °C received doses of 250, 300 and 400 krad. The samples which had been stored at room temperature absorbed doses of 300, 400 and 500 krad. Untreated control samples were also included in the experiments. The irradiated samples in the second series absorbed 200, 250 and 400 krad, resp.

1.3. Storage conditions

A part of the irradiated samples was stored at room temperature (22—24 °C) and the rest in a cellar of 12—14 °C temperature and 85—95% ERH.

1.4. Tests during storage

1.4.1. Determination of spoilage rate. At the commencement of the experiment and at given intervals during storage the number of soft, liquified or mouldy fruit, and the volume of the juice collected in the bottom of the tray were established. After removal of the spoiled fruit and the juice the fruit remaining in the tray was weighed.

1.4.2. Texture determinations. In the first experiment the texture of the fruit was tested as a function of storage time and radiation dose. The measurements were carried out with a "Labor" Penetrometer at the Chemistry Department of the Technical College of the Food Industries, Budapest.

1.4.3. Sensory evaluation. In the second experiment the samples were subjected to triangular test immediately upon radiation treatment and after 1 day storage at room temperature. A panel of 7 judges had to establish which of the three coded samples differed from the other two. Out of the three samples two were treated with the same dose of radiation, the third being the untreated control. Only the sound pieces of fruit were used for sensory evaluation.

1.4.4. Microbiological tests. The qualitative composition of the microflora of both the untreated and radiation treated samples was examined by Mrs P. Nyerges at the Microbiology Department, National Research Institute for Viticulture.

2. Results

2.1. Spoilage

The spoilage characteristics (percentage of spoiled fruit, the volume of juice collected in the bottom of the trays and the weight percentage of sound fruit related to the initial sample weight) of the samples stored at 12—14 °C are shown in Table 1. For the same experiment the spoilage characteristics of the samples stored at room temperature are given in Table 2. The conditions

Table 1

Storage life of Senga Sengana strawberries as affected by gamma radiations (1st experimental series)

A comparison of untreated and irradiated strawberry samples after 1 week storage at 12—14 °C. The table gives the average values at each dose level referring to 2—7 trays containing 0.5 kg strawberries each, and the standard deviations

Radiation dose (krad)	Ratio of spoiled (mouldy liquified) fruits (%)	Juice collected at the bottom of the trays (ml)	Weight of sound fruit relative to the initial amount (%)
0	87 ± 7 a	46 ± 22 a	18 ± 7 a
250	67 ± 18 ab	10 ± 9 b	34 ± 18 ab
300	48 ± 7 bc	5 ± 5 b	50 ± 8 bc
400	40 ± 2 c	5 ± b	56 ± 5 c

The results were evaluated by variance analysis and Duncan test. The average values within the same column, not marked with the same letter, differ significantly at level $P \leq 0.05$. Between average values within the same column marked with the same letter the difference is not significant.

Table 2

Storage life of Senga Sengana strawberries as affected by gamma radiations (1st experimental serries)

A comparison of untreated and irradiated strawberry samples after one week storage at 22—24 °C. The average values at each dose level gained from 1—4 trays containing 0.5 kg strawberries each and the standard deviations, are shown in the table

Radiation dose (krad)	Ratio of spoiled fruit (mouldy and/or liquified) (%)
0	100 ± 0 a
300	69 ± 7 bc
400	56 b
500	76 ± 10 c

The results were evaluated by variance analysis and Duncan test. The average values not marked with the same letter, differ significantly at level $P \leq 0.05$. Between average values marked with the same letter the difference is not significant.

Table 3

Storage life of Senga Sengana strawberries as affected by gamma radiations (2nd experimental series)

A comparison of untreated and irradiated strawberry samples after 2 days storage at 22—24 °C, and after 3 or 6 days storage at 12—14 °C. The table shows the average values and standard deviations obtained from the results of 2—7 samples at each dose level and method of treatment. Each sample tray contained 0.2 kg fruit

Storage temperature °C	Storage time, days	Radiation dose krads	Ratio of spoiled fruit (mouldy and/or liquified) %	Weight of sound fruit compared to the initial amount %
12—14	3	0	81 ± 18 bc	22 ± 18 ef
		200	39 ± 4 gh	67 ± 7 ab
		250	19 ± 11 h	80 ± 14 a
		400	42 ± 12 fg	67 ± 12 ab
12—14	6	0	97 ± 5 a	4 ± 6 g
		200	62 ± 3 de	40 ± 4 cd
		250	50 ± 14 efg	50 ± 12 bc
		400	73 ± 7 cd	32 ± 9 de
22—24	2	0	90 ± 6 ab	13 ± 7 fg
		200	53 ± 8 efg	51 ± 7 bc
		250	51 ± 5 efg	53 ± 5 bc
		400	57 ± 12 ef	47 ± 13 cd

The results were evaluated by variance analysis and Duncan test. Within the same column the average values not marked with the same letter significantly differ at level $P \leq 0.05$. Between average values within the same column, marked with the same letter, the difference is not significant.

of spoilage as observed in the second series of experiments are shown in Table 3. The relative amount of sound fruit left after 5 days of storage at 12—14 °C is illustrated in Fig. 1 (attached). The results obtained in both experimental series are summarized in Fig. 2.

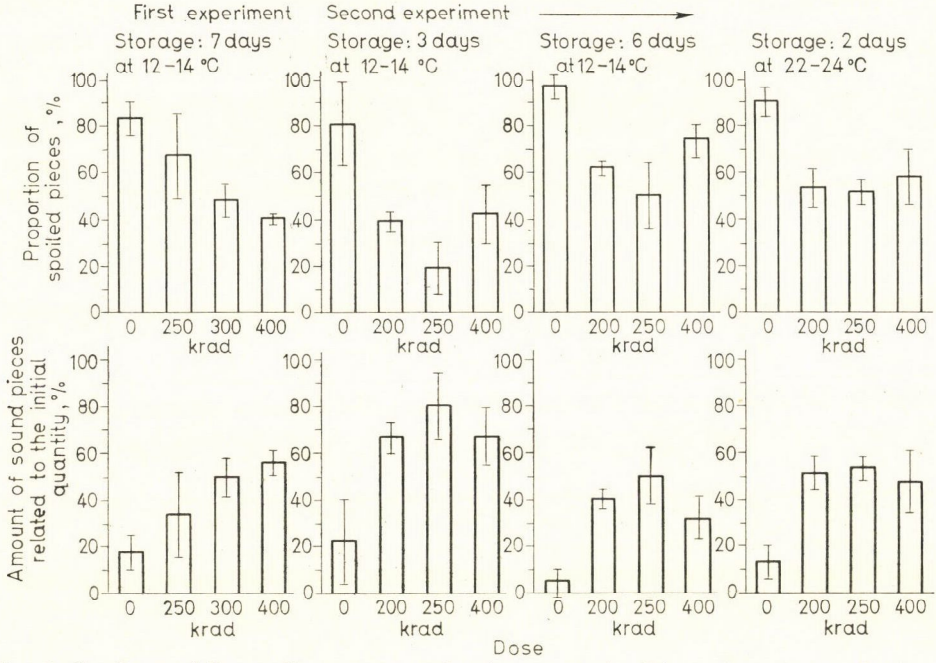

Fig. 2. Spoilage of Senga Sengana strawberries, treated with various doses of gamma radiation and untreated, in the course of the two experiments. The graphs in the top row show the percentage of spoiled pieces and the lower ones that of the sound pieces as a percentage of the initial quantity after the actual storage time. The height of the slabs represents the average value of replicates and the vertical bars the double standard deviation

Table 4

Consistency of Senga Sengana strawberries as affected by gamma radiations and consistency changes during storage (1st experimental series)

Penetrometer tests were carried out on the sound fruit pieces in one tray (15 to 38 pieces), at each storage temperature and dose level. The table shows the average values and standard deviations

	Stored at 12—14 °C		Stored at 22—24 °C	
	0 krad	300 krad	0 krad	500 krad
Initial	34 ± 6	—	24 ± 6	—
After 3 days storage	40 ± 17	52 ± 32	43 ± 21	59 ± 54
After 6 days storage	40 ± 19	45 ± 19	—	—

If compared by Students's t test average values given in the table did not differ significantly at level $P \leq 0.05$.

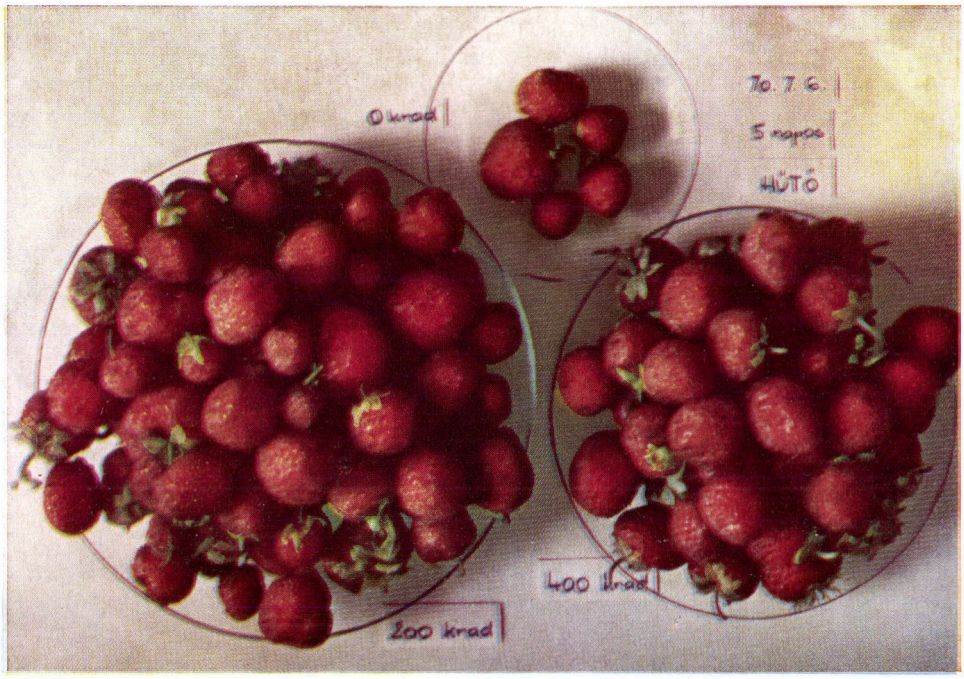

Fig. 1. The relative quantity of strawberries remaining sound after 5 days storage at 12—14 °C: untreated, irradiated with 200 krad and with 400 krad (2nd experimental series)

2.2. Texture of the strawberries as affected by gamma radiation

The results obtained with the penetrometer on the samples of the first experimental series are summarized in Table 4.

2.3. Sensory evaluation

Sensory evaluation of the samples of the second experimental series was carried out immediately upon irradiation and after one day of storage at room temperature. The samples presented first to the panel of 7 judges for triangular test, were those irradiated with 400 krad and the control. In the second test the samples, irradiated with 200 krad, were compared to the control. In both cases the two identical samples were the irradiated ones. The task of the judges with the triangular test was to establish which of the three samples, presented simultaneously, differed from the other two.

Since only 2—4 of the seven judges were able to tell the control from the other two samples, evidently there was no significant difference between the sensory quality of the irradiated and the untreated samples. (The probability of the correct differentiations being not random, is below 5% [KRAMER & TWIGG, 1962].)

2.4. Microbial flora of the strawberries as affected by irradiation

The microbial flora of 8—30 fruit pieces was examined by Mrs Nyerges in the National Research Institute for Viticulture. This was done on the visibly not spoiled control samples and the samples treated with 250 krad in the first experimental series. The dominant microorganisms found on the samples not exposed to radiation were mould strains belonging to the *Rhizopus* genus and various yeast strains (present on about 60% of the fruit pieces). About 23% of the microbial flora on the untreated samples consisted of *Mucor* strains which is a rather high rate. A mixed yeast flora was shown to be present on every piece of irradiated fruit as well, whereas moulds of the *Rhizopus* genus were observed only on about 15%. It should be noted that BERAHA and co-workers (1961) found that the germination of the spores of *Rhizopus stolonifer* could be inhibited only with treatment between 500 and 1000 krad.

3. Conclusions

3.1. Keeping quality of strawterries as affected by irradiation

As shown in the experiments, the spoilage loss of Senga Sengana strawberries in the 3—7-day period between harvesting and marketing can be reduced by treatment with 200 or 300 krad gamma radiation doses to one

half, respectively one fourth. Increasing the radiation treatment above 300 krad did not improve the results, on the contrary, a slight increase of loss was observed in the second experimental series as compared with the optimum obtained with 250 krad treatment.

The present observations are in good agreement with the conclusions of the earlier experiments (FARKAS & KISS, 1967; KISS et al., 1967) when it was established that treatment with a radiation dose of 200—300 krad increased the storage life of strawberries three-fold. These results support the findings of CLARKE (1959) and BERAHA and co-workers (1961). The comparison of the experiments proves at the same time that the differences in the microbial flora and physiological state of the fruit, the efficiency of treatment or the optimal doses may vary from lot to lot. American authors for instance (EUKEL & HUBER, 1959; KETCHUM et al., 1965) consider 200 krad as the optimum dose level. Under the conditions of the present experiments 250—300 krad proved to be more efficient. Soviet authors (KOVALSKAYA et al., 1961) suggested in an earlier publication 400—600 krad dose, because 200 krad proved efficient only when not too ripe strawberries of good condition were treated. KUDRYASHOVA and co-workers (1967) found the efficiency of radiation treatment to be dependent to a certain degree on the dose rate.

3.2. Sensory quality of the irradiated strawberries

The triangular tests of the experimental samples showed that irradiation even with 400 krad exerted no detrimental effect upon the sensory quality of Senga Sengana strawberries. Similarly good results were reported by KOVALSKAYA and co-workers (1961) about the radiation treatment of various Soviet strawberry varieties with 200—600 krad. In England detrimental effects were observed with radiation doses above 500 krad (CLARKE, 1959), for instance discolouration, aroma changes, etc.

Earlier experiments, among them those carried out in cooperation with Soviet researchers proved that the vitamin C content of strawberries was practically not affected by radurization (only by 10—15%) (KOVALSKAYA et al., 1961; KETCHUM et al., 1965; KISS et al., 1967).

Because of the high scatter of the results, no significant differences could be demonstrated between the consistencies of treated and untreated strawberry samples when tested with penetrometer at the Chemistry Department of the Technical College of Budapest for the Food Industries. However, comparing the data as seen in Table 4, a tendency of increasing softening with increasing radiation doses is observed. This observation may be in accordance with the fact that occasionally a lower radurizing effect is achieved with 400—500 krad then with 250—300 krad. It is possible that high radiation doses reduce the resistance in strawberries to moulds, partly by the deleterious effect

upon the texture of the fruit. SOMMER et al., (1968) consider the increase of radiation doses above 200 krad inexpedient because of their softening effect. They found that the dose requirement may be reduced to 100—150 krad by exposing the strawberries prior to irradiation to a mild heat treatment, *i.e.* +40 °C for ten minutes thereby sensitizing the fungi to radiation treatment.

Literary data attribute the spoilage of strawberries mainly to *Botrytis cinerea* and various strains of the *Rhizopus* and *Hormodendron genera* (SALUNKHE, 1961; ANON, 1965b; BARKALAI-GOLAN et al., 1967). It is interesting to note that in the experiments here described Botrytis strains formed no more than 4% of the microbial flora, which is a rather small proportion compared to the 60% frequency of *Rhizopus* strains dominating the microbial flora of Senga Sengana.

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THE INFLUENCE OF IONIZING RADIATION ON THE RIPENING AND STORAGE LIFE OF SOME TROPICAL FRUITS

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Investigation of papaya. The experiments were carried out with papayas of Hawaiian variety which had been irradiated with or without pretreatment. Respiration rate and vitamin C content were determined in samples irradiated with radiation doses of 30, 50, 75 and 100 krad, respectively. In the storage experiments the effect of radiation dose on ripening was studied as function of the time of irradiation and of storage temperature (15°, 18°, 22 °C) and the ripening and rotting indices were evaluated. The tests extended to the investigation of the radiation resistance of fruit in different stages of ripeness.

Investigation of the correlation between storage temperature and radiation dose revealed that at 15 °C papaya loses its normal ripening properties and optimum storage conditions were established as 18 °C and an average of 85% of relative humidity.

A certain change was observed in the organoleptic properties of the irradiated fruit. For an improved storage life with preserved quality a storage temperature of 18 °C and irradiation with 50 and 75 krad radiation dose were found to be the most favourable.

Investigation of mango. Mango of Okrong variety was investigated. The tests extended to the weight loss, changes in the vitamin C content and in the organoleptic properties of fruit irradiated with 10, 20, 40, 50, 60 and 80 krad, respectively, as well as to the determination of the ripening and rotting indices. In course of the storage temperature experiments the irradiated and control fruits were stored at 15°, 18° and 22 °C, and it was found that a temperature of 18 °C and a radiation dose of 40 krad will lead to the most favourable organoleptic and storage properties.

There was no significant difference in the vitamin C content of irradiated and control fruits.

Statistical analysis of the results of organoleptic tests showed no significant difference between the samples irradiated with 40 krad and the controls.

Investigation of rambutan. Rambutans of Bangyeekan and Pink varieties were investigated. In the course of the experiments texture changes, weight losses, changes in vitamin C content, total acidity, changes in reducing sugar content and in the organoleptic properties were determined after irradiation with 10, 20, 30, 40, 50, 60 and 100 krad radiation doses.

It appeared from these tests that from the aspect of radiation preservation the Pink variety had far more favourable properties than the Bangyeekan variety.

Higher radiation doses (e.g. 100 krad) are more favourable from the aspect of extended storage life of rambutan than lower doses.

After 8 days storage the weight loss of samples which had been irradiated with 100 krad was 15% less than that of the controls.

Radiation doses had no effect on the vitamin C content and reducing sugar content of the rambutan samples. A slight decrease in titratable acidity was found in the stored irradiated rambutan samples. The rotting index of the control sample of the Pink rambutan variety was 40% after 10 days and 100% after 16 days, while after 18 days the rotting index of samples irradiated with 50 krad was only 50% and of those irradiated with 60 krad not more than 30%.

Longan investigation. Longan of Doh Yod Khao variety was investigated. In the course of the experiments weight loss, changes in texture, vitamin C content, reducing sugar content and changes in acidity were determined. The effect of treatment with various radiation doses (30, 60, 80, 100, 150, 200 and 250 krad) was evaluated as the function of the rotting index.

The most favourable change in texture was observed on samples irradiated with high doses and then stored. No significant difference was found between the reducing sugar contents and acidity values as function of the storage period. During 15 days storage at 18 °C the rotting indices reached, in case of low radiation doses, 100%, while samples irradiated with 150 and 200 krad, respectively, and stored for 30 days were found to show rotting indices of not more than 60 and 80%.

Radiation doses of 100 and 200 krad failed to reduce significantly the vitamin C content of irradiated and stored longan samples.

No difference was found in the organoleptic properties of irradiated and control samples.

Finally, with respect to the experiments with papaya, mango, rambutan and longan, it is pointed out that no comparative data are available from the literature on rambutan and longan, since this appears to be the first time when the effect of ionizing radiation on these fruits has been studied.

The efforts to ensure food supplies include besides increased production, with growing emphasis, the protection of goods produced. Works which account for the satisfaction of demand in developing countries are particularly important from this aspect. Because of its versatile applicability and manifold effects ionizing radiation has come into the centre of attention. A reduction of food losses (caused mainly by insects and microorganisms) estimated by FAO to amount to about 20% of world supplies, would be an outstanding result. In works of this type the application of ionizing radiation must not be neglected, since it might be applied to the solution of problems which have escaped all other procedures (VAS, 1969).

The importance of investigations into the utilization of ionizing radiation is demonstrated by the fact that research on the radiation treatment of foodstuffs is now being carried out in a totality of 48 countries, including Thailand where extensive and systematic research is in progress to use ionizing radiation for influencing the ripening process of tropical fruits and to improve the storage properties of sea fish.

One of the authors (József Kovács) of the present paper has taken part as an expert of the International Atomic Energy Agency (IAEA) in the investigation of the topical problems in Thailand where the experimental work has been performed in the Biology Department of the Office of Atomic Energy for Peace.

The research programme was drawn up on the basis of the following aspects:

Investigation and determination of the conditions of harvesting, storing and preserving various tropical fruits of equal importance in Thailand and the neighbouring countries. Since there is no single definite method which alone would be suitable for the solution of all problems, a deeper insight into a

treatment of complex effect, such as irradiation, and the elaboration of the corresponding technology would be of particular importance.

It was our aim to study the ripening and changes during storage of various tropical fruits, such as papaya, mango, rambutan and longan:

— with fruits of different degrees of ripeness and of different variety;
— after irradiation with various doses;
— after various pretreatments;
— as a function of storage temperature.

To follow up the biochemical and physiological changes occurring as an effect of radiation treatment and during storage we have chosen physical and chemical, as well as sensory methods which were deemed appropriate on the ground of literary data and according to our own experience both for the experiments and for the evaluation of the results.

We wish to point out however, that in applying ionizing radiation our intention was not to replace well known and in certain cases satisfactory preservation methods, but to work out a useful combination with the latter.

According to the literature on various irradiation experiments carried out with *papaya* (Carica papaya), radiation may induce accelerated ripening just as it may cause the retardation of the ripening process. It is impossible to give a current general estimation of all effects observable after irradiation (MAXIE & SOMMER, 1968; BIALE, 1950, 1960). In case of fruits in the climacteric group the delay in ripening will depend on the time when irradiation is applied.

ROSS and BREWBAKER (1965) reported on excellent results with Hawaiian type papaya after irradiation. Radiation effect was highly significant from two aspects. Combined with warm water treatment 75 krad retarded ripening and fungal spoilage thereby about doubling shelf life at room temperature. At the same time, this dose is more than three times higher than the 21 krad needed for the destruction of larvae and insects, so that in this way the quarantine of imported fruits into the United States may be avoided.

MAXIE and STALLMAN (1963) too, irradiated the Hawaiian papaya variety with 100, 200 and 300 krad and stored the irradiated fruit at 20 °C and 90—95% relative humidity. Evaluating taste and appearance they found that after irradiation with 200 and 300 krad ripening was much slower than that of the controls and the off-flavour following radiation treatment was not observed.

Mango (Magnifera indica, Linn) is a tropical fruit which ripens in a relatively short time and deteriorates rapidly, so that here the elaboration of a suitable method of storage is particularly important. The combined application of cold storage and various desinfectant methods has failed to bring the desired result.

Irradiating the Alphonso variety of mango DHARKAR and SREENIVASAN (1966) observed that shelf-life could be best prolonged by treatment with 25 krad (shelf-life prolongation of 6 days), while the results obtained with 12, 50 and 75 krad were unsatisfactory.

SOLANAS and DARDER (1966) investigated in Venezuela the Bocado variety mango and found that the effect of 1 krad doses was sufficient to improve shelf-life at 18 °C and 80% relative humidity.

A somewhat lower radiation sensitivity was recorded for the Carabo variety mango growing in the Philippines.

MUMTAZ, FAROOQI and AMIZ (1968) irradiated ripe green hard Desi variety mango with 10, 20, 25 and 30 krad at room temperature. The best result in retarding was observed at 30 krad.

Mango was radiation treated with surprisingly high doses (100 and 250 krad) in the Puerto Rican Nuclear Centre.

In Thailand the preliminary experiments were begun in 1966, with Okrong variety mango using 10, 20 and 40 krad doses. Relatively good results were obtained with 40 krad. In 1967 fruits in different stages of ripeness were exposed to treatment with 20, 40, 50, 60 and 80 krad and the irradiated fruits were stored at 21 °C. The best storage results were obtained with mango harvested at the age of 104 days, whether the samples were irradiated (with 40 krad) or not.

On the basis of these data and of the degree of ripening the results of fruit tests were checked and extended to treatment with 10, 20, 30, 50, 60 and 80 krad doses.

Rambutan (Nephelium lappaceum, Linn.) is a tropical fruit of high nutritional value. In Thailand it is one of the traditional fruits grown mostly in southern parts of the country. It blossoms in January and February and ripens about five to eight months later.

There are no literature data on the radiation treatment of rambutan, thus we were the first to investigate the radiation sensitivity of the fruit. The oldest variety grown in Thailand is the Bangyeekan, but the Pink, Bangyeekan Penang and Che-mong varieties are also well known.

The irradiation experiments were performed with a twofold aim: partly to study the effect of ionizing radiation on the quality of the fruit as a function of dose and partly to investigate the influence of the variety on the results.

It should be pointed out that with respect to radiation treatment and variety the reports in the literature are not unambiguous, and we have therefore devised our experiments in such a way as to be able to ascertain reliably the effect of the variety.

Longan (Euphoria longana, Lank) is a tropical fruit with very pleasant taste, similar to rambutan and lychee. In Thailand about seven or eight varieties are being grown currently, mainly in the northern part of the country.

The season of longan is July and August. From blossoming to full ripening of the fruit takes about five months.

There are no data in the literature on the radiation sensitivity of longan, thus special experiments had to be devised for the determination of radiation doses influencing the basic taste and texture.

1. Materials and methods

1.1. Preparation of the samples

In the papaya experiments the Hawaiian variety grown in Thailand in the Pak-Chong district was used.

The freshly gathered fruit in different stages of maturing was transported into the laboratory in lined boxes, protected from mechanical injuries and was stored there at temperatures between 18° and 20 °C up to the time of the experiment.

Generally the fruits were classified according to size and stage of ripening within 24 hours after their arrival and groups of 6 to 15 fruits were prepared for radiation treatment. In order to remove surface dirt the fruits were washed with a dilute permanganate solution (1 : 10,000) for 15 to 20 minutes.

To study the effect of various other treatments heat treatment with hot water (in water of 75° to 80 °C for a few seconds) and wax coating were used followed by the irradiation of the fruits.

The fruits were radiation treated with Cobalt-60 Gammacell at 20 °C, using an about 8000 rad per minute dose rate.

The doses were: 30, 50, 75 and 100 krad, respectively.

During treatment the irradiated and control fruits were kept at identical temperatures and were placed at the same time in the various storerooms where they were stored at 15°, 18° and 22°, respectively, and at 85 to 95% relative humidity.

For the respiration rate tests besides the samples of approximately the same size and weight also control and irradiated specimens obtained by slicing some larger papaya fruits were used. The samples were placed in 10 litre desiccators.

For the determination of the vitamin C content specimens were prepared whose homogeneity was ensured by the slicing of some larger fruits. The specimens were placed into bags prepared of saran foil sealed under vacuum.

The mango experiments were carried out with the green Okrong variety grown in Thailand in the Amphur Dumneunsaduak district.

The freshly picked fruits were delivered to the laboratory where they were classified according to the degree of ripeness. Groups of 50 to 100 fruits were subjected to radiation and stored.

The doses were: 10, 20, 30, 50, 60 and 80 krad, respectively.

The specimens were stored in air conditioned rooms of 18 °C and 85 to 95% relative humidity.

Respiration rate was determined on samples consisting of the same number of fruit of about the same size and the evolved carbon dioxide was measured at 20° to 22 °C.

For organoleptic tests and for the determination of the vitamin C content special groups were formed from the stored fruits.

The rambutan tests were carried out on the Bangyeeken and Pink varieties grown in the south eastern districts of Thailand. The freshly picked fruits were transported into the laboratory in baskets, where they were divided into groups of 100 to be irradiated and stored.

The doses were: 10, 20, 30, 40, 50, 60 and 100 krad, respectively. The experimental material was stored at 18 °C and 85 to 95% relative humidity.

Special groups were formed for the organoleptic tests and for the determination of the vitamin C content.

The longan tests were performed on the Doh Yod Khao variety grown in the northern district of Chiengmai in Thailand. The harvested longan was carried in bamboo baskets into the laboratory where an about 1 cm long stem was left on the fruit. Each experimental batch contained 150 fruits.

Longan was irradiated, 24 to 36 hours after having been picked, with 10, 30, 60, 80, 100, 150, 200 and 250 krad doses, respectively.

In the preliminary tests the experimental material was stored at 15°, 18° and 23 °C, in the systematic storage tests at 18 °C and 85 to 95% relative humidity.

1.2. Methods

In course of the storage tests the ripening and rotting indices of the fruit were observed daily by noting changes in appearance and the apparent symptoms of ripening and rotting. For the determination of the ripening index surface colour changes (changes in the texture of the flesh of fruits used in other tests) were observed. The detectable symptoms of ripening were evaluated by scoring from the percentage ratio of the surface for each fruit and each treated lot (calculated for 20 to 100 fruits). In this way the "ripening index" meant in each case a value expressed in percentage of the maximum attainable score for ripeness (full ripening) for the sample lot investigated in a given experiment.

The rotting index was determined in a manner similar to the determination of the ripening index, scoring in this case the signs and degree of rotting (browning, moulding, desiccation, other discolourations).

Weight losses were determined by checking daily the weight of the entire stored material.

In the respiration measurements (in the case of papaya and mango) the carbon dioxide formed during the ripening process was determined according to VOGEL's method (1964) by parallel measurements performed twice daily.

Fruits qualified as ripe were subjected to organoleptic tests by a panel of ten. The method involved the use of a 9 point scale combined with ranking. Taste and aroma were tested using a scale corresponding to the following terms: 9 — excellent, 8 —very good, 7 — good, 6 — fair, 5 — neutral to the judge, 4 — poorer quality, 3 — medium poor, 2 — poor, 1 — extremely poor.

The scores obtained as the result of sensory tests performed by this method were evaluated by means of variance analysis (JELLINEK, 1964; PEARSON & BENNETT, 1942; DIXON & MASSEY, 1951).

Sensory tests were carried out by the judges in a room specially equipped for the purpose. Three judges worked at the same time, separated from each other.

Changes in vitamin C content were determined in the control and irradiated samples, prepared in the same manner, by means of Tillmann's titration method (JACOBS, 1959).

The reducing sugar content was determined by means of the titration method of LANE and EYNON using Fehling's reagent described by NUNSON and WALKER (cit. JACOBS, 1959).

The texture tests were performed with a dial gauge provided by a special conically shaped head for penetration, worked out in the course of our own experiments. The data obtained with the modified dial gauge were satisfactorily comparable and could be calculated with the help of an empirical scale.

Changes in acidity were studied by titration (cit. JACOBS, 1959).

2. Results and conclusions

2.1. Evaluation of the papaya tests

2.1.1. Evaluation of the results of storage tests. The effect of storage temperature was tested in the temperature range of practical importance, namely at 15°, 18° and 22 °C.

Besides the changes in the ripening index the rotting index, too, proved that storage temperatures 15° and 22 °C were not suitable not even for the irradiated samples. Though there was a certain difference in the quality of the control and the irradiated samples at 15 °C and 22 °C, however this could not be evaluated from the point of view of storage or of the preservation of fruit texture.

It appears from the experimental data of samples in two different stages of maturity, used as control and irradiated with 50 krad, respectively, and

stored at 15 °C that unripe fruit protected from light will not ripen at this temperature. In the case of less ripe papayas irradiation stimulated ripening, manifested in changes of colour and texture, but in the experimental period of time the effect of irradiation could not be evaluated unequivocally.

Papayas of identical maturity show at 18 °C storage temperature quite clearly the effect of irradiation as a function of the dose. Lower doses stimulated ripening, but at 60 krad there was already a significant decrease in the ripening index as compared to that of the control. When a dose of 70 krad was applied the ripening index was still not more than 50 % after 27 days of storage, compared to the 100 % found for the control.

Table 1

Average ripening period of control and irradiated papaya, days

Storage temperature °C	0	30	50	100
	krad doses			
15	13	—	17	—
18	40	46	33	—
22	16	—	25	18
22 (unripe)	20	—	25	27

Average standard deviation: 11.7 days
Least significant difference: 4.4 days

In Table 1 the average ripening time (the time required for reaching a 50 % ripening index) of papayas irradiated with various doses and stored at various temperatures, is given.

In the curves for 15 °C and 18 °C storage temperatures the data of initially ripe fruit are shown, since papayas which were initially unripe failed to ripen to any estimable degree within the period of the test and were therefore not included in the Table.

Unripe fruit stored at 22 °C ripened at a rate which made it possible to evaluate the experimental data of initially unripe fruit at the end of the storage test, thus they are given in Table 1.

It appears from Table 1 that both the dose and the storage temperature have a non-linear effect on the ripening of papaya. A storage temperature of 18 °C seems to produce an outstanding maximum from the aspect of prolonged average ripening time. The effect of the dose has a maximum at this temperature, but the temperature dependence of the dose with maximum effect cannot be established unequivocally from the experimental data. It appears from the investigation of the relationship between initial maturity and the

radiation dose with maximum effect that in the case of unripe fruit this dose shifts in the direction of higher values.

A parallel investigation of the ripening and rotting indices is the fundamental requirement of the adjustment of appropriate storing conditions (Table 2).

The effect of the dose as a function of the time when irradiation is applied can be measured only from the more favourable trend of the ratio of the ripening and rotting indices. From this aspect only the trend of the data pertaining to fruits stored at different temperatures can be compared, since it is impossible to ensure fruit in exactly identical stage of maturity for each experimental series. It is possible, however, to achieve some minor fluctuation of homogeneity in each experimental series necessary for the estimation of certain dose effects (Fig. 1).

In case of untreated and treated fruits stored at 22 °C, rotting set in within a remarkably short time, compared to storage at 15 °C and 18 °C. This qualitative change was manifest both in the loss of water due to evaporation and in extensive surface mould growth. Relatively acceptable storage results were found at 50 krad doses.

Table 2

Ripening and rotting indices of papaya stored at 18 °C (%)

Period of storage, days	Control		30 krad		50 krad		75 krad	
	Ripen.	Rotting	Ripen.	Rotting	Ripen.	Rotting	Ripen.	Rotting
7	—		4.2		—		—	
10	4.2		4.2		4.2		4.2	
17	4.2		8.4		12.5		4.2	
18	4.2		4.8		12.5		4.2	
19	4.2		8.4		16.7		4.2	
20	4.2		8.4		16.7		4.2	
21	4.2		8.4		16.7		4.2	
24	16.7		8.4		25.0	16.5	4.2	16.5
28	25.0		15.0	16.5	35.0	16.5	12.5	67.0
29	35.0	16.5	20.0	16.5	55.0	16.5	12.5	67.0
30	35.0	16.5	20.0	16.5	55.0	50.0	—	100.0
32	35.0	16.5	20.0	16.5	58.5	50.0	—	100.0
36	35.0	16.5	20.0	16.5	58.5	50.0	—	100.0
38	35.0	16.5	20.0	16.5	58.5	50.0	—	100.0
39	35.0	16.5	25.0	50.0	58.5	50.0	—	100.0
43	50.0	50.0	50.0	67.0	58.5	67.0	—	100.0
45	50.0	50.0	50.0	67.0	62.5	67.0	—	100.0

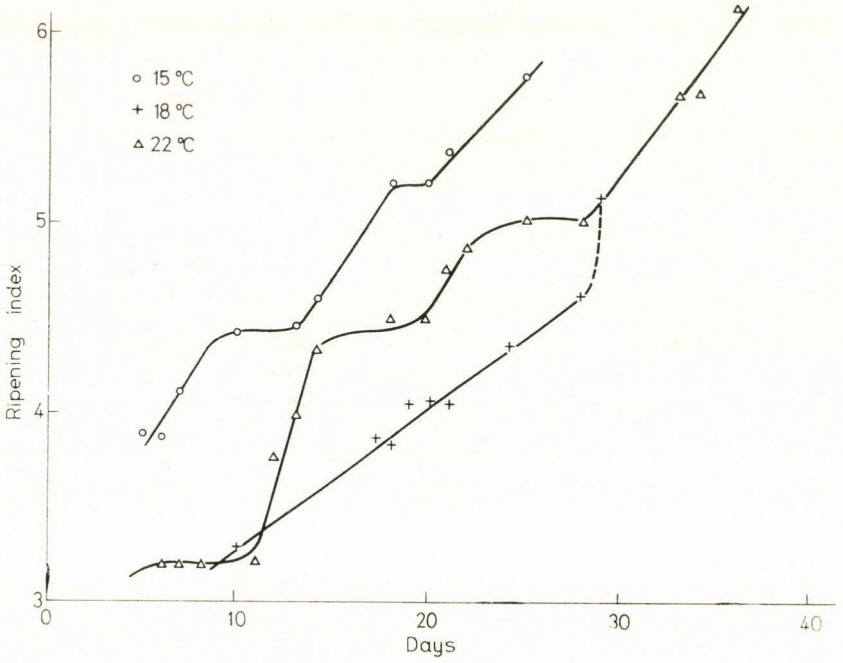

Fig. 1. Ripening index values of papaya irradiated with 50 krad and stored at 15°, 18° and 22 °C

The effect of wax coating and of hot water pretreatment appears from the data in Table 3.

Table 3

Storability of papayas subjected to radiation combined with wax coating and hot water treatment

Sample	Period of storage, days		
	Wax	Hot water treatment+wax coating	
	stored at 18 °C	stored at 18 °C	stored at 25 °C
Control	10	12	10
40 krad	14	17	14
50 krad	12	19	12
70 krad	14	17	—

The storage life and quality of fruit of identical maturity and receiving a pretreatment in hot water and wax coated, was found most favourable when irradiated with 50 krad.

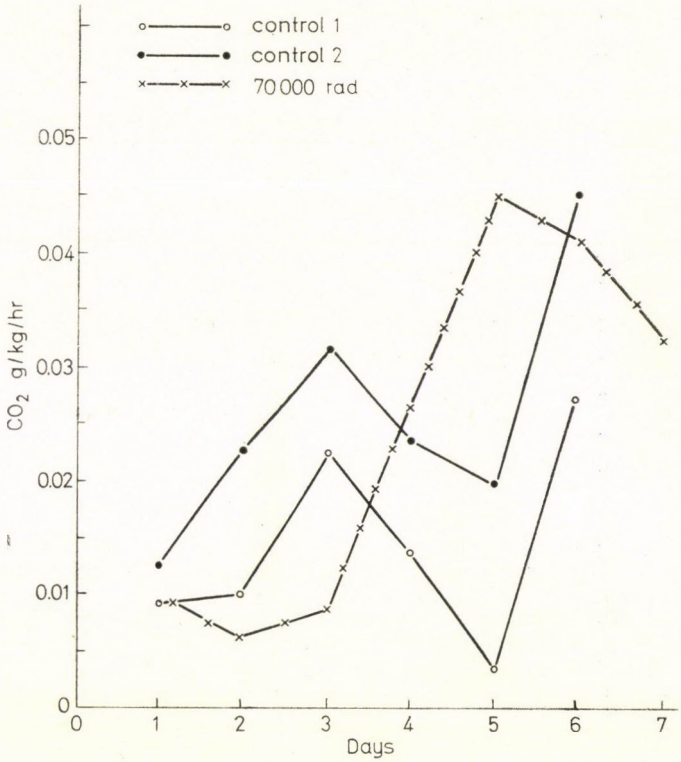

Fig. 2. Respiration degree of control and radiation treated papaya

2.1.2. Evaluation of the results of respiration degree determinations. Figure 2 shows quite clearly the measurable difference in the carbon dioxide evolution of untreated samples and of samples irradiated with 70 krad.

Irradiation has a significant delaying effect upon ripening. As a complement to the radiation effect tests, the quantity of carbon dioxide evolved by samples irradiated with 50 and 100 krad, respectively, was measured parallel to the previous tests in the course of comparative investigations (Fig. 3).

The results of the comparative tests showed no characteristic difference either with respect to the quantity of evolved CO_2 or its changes in time between the two samples irradiated with different doses.

2.1.3. Evaluation of the results of sensory tests. When evaluating the results of sensory tests it has to be pointed out that the findings of judges who at repeated tests gave the same score were used for evaluation.

Investigating the effects of treatments as functions of time from the technological aspects, the sensory tests were carried out at various times depending on the ripening of the fruits and on the changes appearing on their surface. Considering the fact that we intended to investigate primarily the

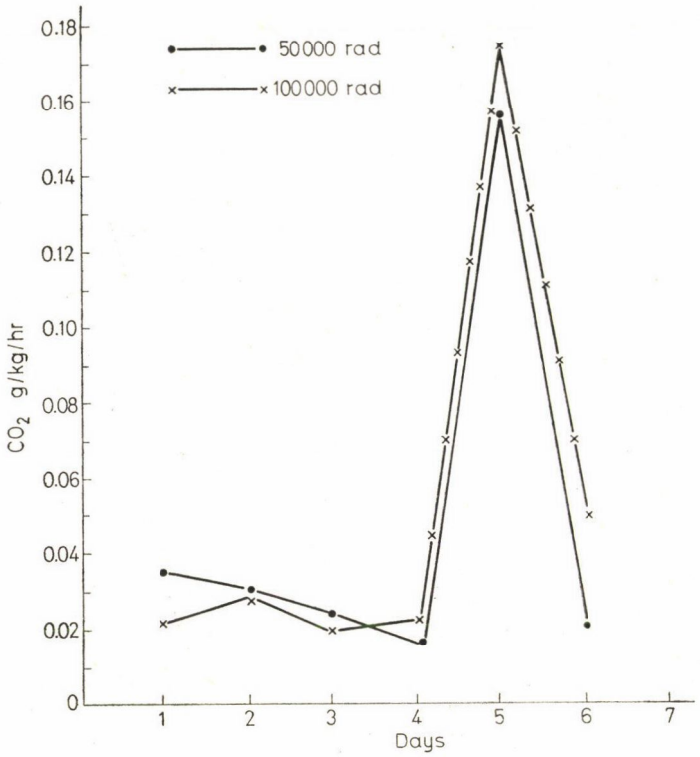

Fig. 3. Respiration degree of irradiated papaya

Table 4

Comparison of the organoleptic properties of control and radiation treated papaya samples

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	140	40		
Treatments	44	2	22.00	19.45
Persons	52	5	10.40	9.22
Interaction	20	10	2.00	1.77
Residue	26	23	1 13	

Storage period: 12 days

1st sample: control, stored at 18 °C $\bar{X}_1 = 3.28$
2nd sample: 50 krad, stored at 18 °C $\bar{X}_2 = 5.00$
3rd sample: 50 krad, stored at 20 °C $\bar{X}_3 = 2.4$

Table 5

Comparison of the organoleptic properties of irradiated papaya samples

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	170	59		
Treatments	65	2	32.5	42.2
Persons	68	9	7.55	9.79
Residue	37	48	0.77	

Storage period: 13 days

1st sample: 30 krad, stored at 18 °C $\overline{X}_1 = 3.3$
2nd sample: 50 krad, stored at 18 °C $\overline{X}_2 = 4.16$
3rd sample: 75 krad, stored at 18 °C $\overline{X}_3 = 5.09$

effect of treatments, we determined the interaction between treatment and man without analyzing it in detail since the two components of deviation are related mainly to physiological problems.

The beneficial effect of storage at 18 °C and treatment with 50 krad appears unambiguously from the data of Table 4.

In course of the sensory tests of samples irradiated with 50 krad and stored together with the controls at 18 °C, the judges found on the 12th day that these are unambiguously better than similar samples stored at 20 °C.

According to the comparative ranking of samples treated with different doses (Table 5) the trend of changes to be expected during storage tests is quite clearly noticeable.

Table 6

Comparison of the organoleptic properties of control and radiation treated papaya samples

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	37	27		
Treatments	8	2	4.00	9.32
Persons	5	6	0.83	2.00
Interaction	21	12	1.75	4.09
Residue	3	7	0.43	

Period of storage: 16 days

1st sample: control, stored at 18 °C $\overline{X}_1 = 5.08$
2nd sample: 30 krad, stored at 18 °C $\overline{X}_2 = 6.13$
3rd sample: 75 krad, stored at 18 °C $\overline{X}_3 = 5.28$

Table 7

Comparison of wax coated and uncoated papaya samples

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	206	43		
Treatments	52	3	17.3	9.5
Persons	67	4	16.8	9.2
Interaction	43	12	3.58	1.86
Residue	44	24	1.83	

Period of storage: 29 days

1st sample: control (wax coated), stored at 18 °C $\bar{X}_1 = 6.25$
2nd sample: 30 krad (wax coated), stored at 18 °C $\bar{X}_2 = 6.7$
3rd sample: control (without wax), stored at 18 °C $\bar{X}_3 = 4.9$
4th sample: 75 krad (without wax), stored at 18 °C $\bar{X}_1 = 3.58$

In the dose range applied in this series of experiments no off-flavour was observed.

From the data in Table 6 a slightly significant difference (0.05 %) appears between the controls and certain irradiated samples.

Table 7 contains the results of the sensory evaluation, on the 29th day of storage, of samples partly wax coated, partly without coating and given a 30 or 70 krad treatment, respectively. It is evident from these data that the combined treatment is extremely more favourable.

Though treatments have a significant effect, there might be a considerable difference between the scores given to samples by different judges as shown also by the high significance level.

Table 8

Vitamin C content of control and radiation treated papaya samples (mg/100 g)

Period of storage	Control		30 krad		50 krad		75 krad	
	original	dry	original	dry	original	dry	original	dry
Ripe papaya								
5 days	58.5	585.0	52.4	560.0	37.3	377.0	48.1	460.0
7 days	42.5	—	42.5	—	42.5	—	41.2	—
Unripe papaya								
5 days	35.0	318.0	29.5	268.0	41.6	378.0	37.2	343.0
7 days	34.4	—	31.6	—	35.8	—	27.4	—

2.1.4. Evaluation of the results of vitamin C determinations. In order to avoid the inhomogeneity of samples used in the determination of the changes in vitamin C content specimens obtained by slicing some larger fruits were also used. In testing the effects, of course, both ripe and less ripe papaya samples were used. The data in Table 8 show the difference in vitamin C content of the controls and of samples irradiated with 30, 50 and 75 krad, respectively. The data show an increase in vitamin C content in the unripe irradiated samples. The vitamin C content of the ripe samples decreased in the first five days of storage and was constant after the 7th day.

Repeated comparative tests in dependence on storage time showed similar results after 12, 18 and 24 days (Table 9).

Table 9

Changes in the vitamin C content of papaya during storage

Period of storage days	Vitamin C content of papaya, mg%		
	control	40 krad	50 krad
12	62.9	—	52.4
18	64.5	83.0	82.2
24	51.5	—	52.0

2.2. Evaluation of the mango tests

2.2.1. Evaluation of the results of the storage experiments. From the results of the storage tests of untreated and irradiated mango samples two important conclusions can be drawn. One is that when unripe green fruit is picked at a certain time the irradiated samples of such fruit will need a significantly longer period of time (about 1.5 times longer) for ripening than the controls. The second and perhaps from the aspect of judging the effect of treatment the more important fact is that in a state corresponding to 50% ripening index, and generally in the state of development preceding full ripeness (100% ripening index), the distribution of the samples is fundamentally different in the control and irradiated fruits (Table 10).

It appears from Table 10, and Table 11 which sums up the results of the variance analysis of the data of the preceding Table, that changes due to radiation take place in the majority within 28 days from the beginning of storage. Investigation of the relationship between ripening degree and radiation effect has revealed the following facts:

On mango of 117 days initial ripening the dose had no significant effect.

Table 10

Average ripening period of differently treated mangoes in different stages of ripeness

Maturity, days	Dose, krad				
	0	20	40	50	60
Series 106 days, I	11	16	15.5	16	19.5
Series 106 days, II	11	13	14.5	14	14.5
Series 117 days	5.5	—	—	—	6.5

Average standard deviation: 6.0 days
Least significant difference: 1.3 days

The ripening period of mango picked on the 106th day was gradually prolonged by irradiation (Fig. 4), but the results of the experiments provide not enough information for the extrapolation of these stepwise changes. The position of the second step might only be clarified by using doses considerably higher than the 60 krad which is adequate for practical purposes.

Comparison of the ripening period of fruits picked at various times (at various degrees of ripeness) has shown that without irradiation the fruits picked earlier ripen more quickly than the difference in the times of picking. Radiation treatment increases more and more the ripening period of fruits picked in unripe condition and equalizes the differences in the times of picking.

Weight loss tests (Table 12) have indicated that at the beginning of the storage period radiation treatment has no effect on weight loss.

Table 11

Variance analysis of the ripening period tests of mango

Source of variance	Sum of squares (SQ)	Degrees of freedom (DF)	Mean square (MS)
Total	178	1	
106 days	57.6	9	
series	7.1	1	7.1
dose	38.1	4	8.6
series x dose	12.3	4	3.1
117 days dose	0.5	1	0.5 (not significant)
106—117 (maturity)	120.0	1	120.0
Error		~1200	0.35

As a function of storage time, after 18 days the loss of weight of fruits was lower after irradiation with higher doses, but this cannot be considered a significant change.

2.2.2. Evaluation of the results of the respiration rate determinations. The results of the respiration rate tests showed no estimable difference between the results of the control and irradiated samples.

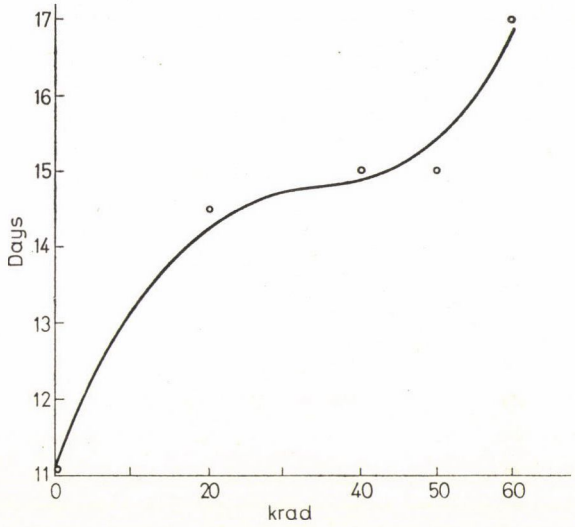

Fig. 4. Average ripening time (of 106 days old mango) vs. radiation dose

Table 12

Weight loss of mango stored at 18 °C

Period of storage, days	Control	20 krad	40 krad	50 krad	60 krad
10	8.3	7.4	8.8	8.9	7.3
11	6.9	8.7	8.3	8.1	8.1
16	7.4	10.2	9.0	11.3	11.5
18	—	—	14.6	14.8	12.9

2.2.3. Evaluation of the results of sensory tests. Investigation of the changes in the organoleptic properties of mango during storage has shown that sensory ranking as a qualitative procedure can be considered only for fruits in a state approximating full ripeness. The sensory tests used for evaluation were performed after 21 and 26 days of storage. It appears from the scores of the 21st day (Table 13) that samples of nearly identical quality were ranked since there is hardly any difference in the average values.

Table 13

Comparison of the organoleptic properties of control and radiation treated mango samples after 21 days storage

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	198	71		
Treatments	42.15	3	14.05	5.26
Persons	10.6	8	1.32	0.48
Interaction	45.75	24	1.9	0.69
Residue	99.5	36	2.76	

Period of storage: 21 days

1st sample: control, stored at 18 °C $\overline{X}_1 = 6.88$
2nd sample: 40 krad, stored at 18 °C $\overline{X}_2 = 6.81$
3rd sample: 50 krad, stored at 18 °C $\overline{X}_3 = 6.11$
4th sample: 60 krad, stored at 18 °C $\overline{X}_4 = 6.27$

It must, however, be stressed that in none of the irradiated samples did the judges notice off-flavour due to irradiation.

In relation to the evaluation of the sensory tests after 26 days storage (Table 14) it must be pointed out that after such a long storage period original control sample was not available.

Consequently we used the control sample of another sample lot (stored for 19 days), prepared parallel to the first series of experiments, whereby our aim was to record any difference compared to a high quality control. The signif-

Table 14

Comparison of the organoleptic properties of control and radiation treated mango samples after 26 days storage

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	423	97		
Treatments	89	3	29.66	16.4
Persons	116	9	12.88	7.12
Interaction	113	27	4.18	2.32
Residue	105	58	1.81	

Period of storage: 26 days

1st sample: control (19 days), stored at 18 °C $\overline{X}_1 = 7.55$
2nd sample: 20 krad, stored at 18 °C $\overline{X}_2 = 5.25$
3rd sample: 50 krad, stored at 18 °C $\overline{X}_3 = 5.52$
4th sample: 60 krad, stored at 18 °C $\overline{X}_4 = 5.05$

icant differences in the treatments can be traced back primarily to this high quality control. Checking of score sheets revealed, however, that various judges gave the various irradiated samples rather different scores. This result is reflected among others also in the interaction: man-treatment.

2.2.4. Evaluation of the results of vitamin C determinations. Changes in vitamin C content appear quite clearly from the data in Table 15; there is a marked increase in vitamin C content during the ripening of certain fruits.

Table 15

Changes in the vitamin C content of irradiated mango (mg/100 g)

Age of fruit when picked, days	Samples	Period of storage after irradiation		
		7 days	14 days	22 days
106	control	15.3	42.5	24.3
106	20 krad	15.5	33.0	28.8
106	30 krad	16.2	32.2	24.1
106	40 krad	14.6	37.0	25.3
106	50 krad	16.6	33.0	27.9
		1 day	4 days	6 days
117	control	23.1	23.2	20.7
117	40 krad	23.5	16.5	19.2

Similarly to papaya, irradiation caused in not fully ripe mango certain increase of the vitamin C content after seven days of storage, while in the later period of storage, after 14 and 22 days, a significant decrease in vitamin C content was observed. 117 days old, ripe mango showed after irradiation and during storage a similar change in vitamin C content. These results indicate a decisive dependence of the vitamin C content on the physiological condition of the fruit.

The favourable results obtained in the storage test, as well as the relationships between the various parameters, have shown in case of mango of all the tropical fruits in our experimental programme most markedly the importance of knowing the degree of maturity.

2.3. Evaluation of rambutan tests

2.3.1. Evaluation of the results of the storage experiments. In the course of storage tests the effect of variety and changes in rotting index were studied on parallel prepared lots of control and irradiated samples. The rotting indices obtained after irradiation and storage of the two rambutan varieties, Pink

Table 16

Average rotting period of Pink and Bangyeekan variety rambutans, days

Variety	Dose, krad						
	0	10	20	30	40	50	60
Pink	13.6	13.8	14.3	14.4	15.1	15.4	16.1
Bangyeekan	13.0	12.0	13.2	12.6	13.0	12.6	13.7

Average standard deviation: 1.6 days
Least significant difference: 0.33 days

and Bangyeekan, (Table 16) and the variance analysis of the data in Table 16 (Table 17) show quite clearly the beneficial effect of higher doses on the quality of the fruit. This beneficial effect is manifest in a prolongation of storage time.

The distribution of the rotting indices of the Bangyeekan variety is not as favourable as that of the Pink variety, but at 60 krad the significantly beneficial effect of the dose could be determined.

The favourable effect of variety appears unambiguously from the changes in rotting index; in general the Pink variety has given more favourable results. To confirm the influence of the variety, tests were carried out in which the Pink variety rambutan was irradiated with 0, 20, 60 and 100 krad doses (Table 18). The experimental results confirmed the findings obtained in the previous series of experiments. The results of treated fruits are unequivocally better as functions of storage time when higher doses are used, and this holds even for longer periods of storage.

It appears further from the experimental results that in case of the Pink variety the average rotting index vs. dose increases according to a curve of second order.

Because of the less favourable behaviour of the Bangyeekan variety the nature of the correlation with dose level is impossible to evaluate; this is shown in Fig. 5.

Table 17

Variance analysis of the average rotting period tests of rambutan

Source of variation	SSQ	DF	MS
Total	17.7	13	
Dose	5.1	6	0.85
Variety	10.8	1	10.8
Dose x variety	1.8	6	0.29
Error		~1000	0.028

Table 18

Rotting indices of variety rambutan

Period of storage, days	Rotting indices, %			
	control	20 krad	60 krad	100 krad
4	1	—	—	1
8	4	4	6	2
10	33	22	19	4
11	42	41	24	5

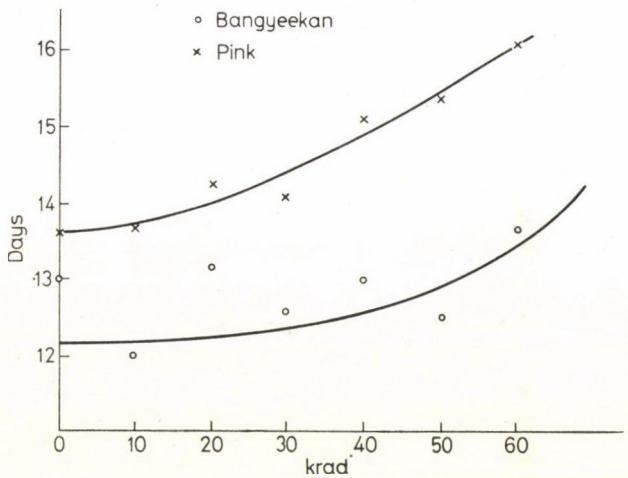

Fig. 5. Storability of Pink and Bangyeekan variety rambutan vs. radiation dose

Table 19

Weight loss of rambutan after 8 days of storage

Treatment	Weight loss in %	
	Pink	Bangyeekan
Control	36.0	33.1
10 krad	34.0	33.3
20 krad	(27.6)	(30.0)
30 krad	33.0	32.7
40 krad	28.2	(28.8)
50 krad	31.0	33.5
60 krad	27.0	31.8

Fig. 5 shows that in the case of the Pink variety the investigated lots of rambutan originate from a homogeneous multitude. Rotting begins after a slight initial rotting period.

Investigation of the weight loss of the irradiated and stored fruits (Table 19) has led to the finding that in agreement with the results of the previous tests weight loss is considerably lower in case of the irradiated samples.

2.3.2. Evaluation of the results of the investigation of texture. Texture was investigated parallel to the storage experiments on the 9th and 13th day (Figs 6 and 7) showing a significantly favourable effect of the higher doses

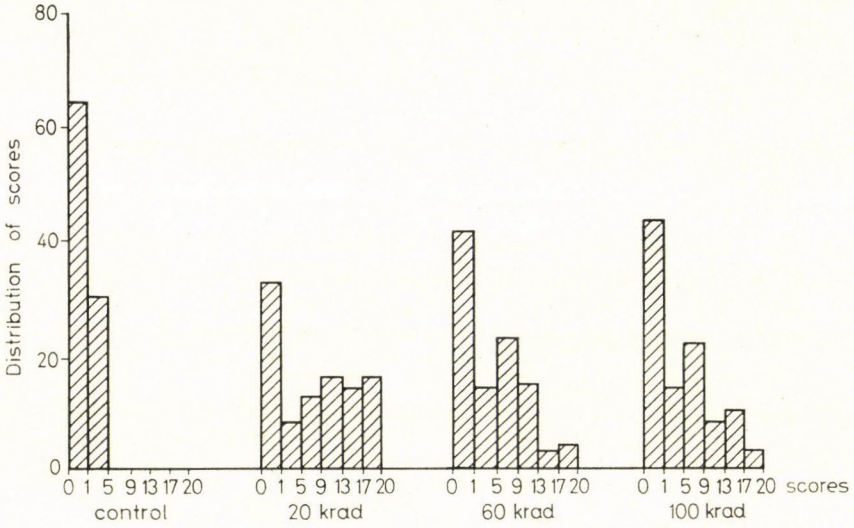

Fig. 6. The texture of rambutan after storage at 18 °C for 9 days

While 100% of the samples untreated and irradiated with 20 and 60 krad possessed after 13 days storage a texture resistance within 0.1 division of the scale, samples treated with 100 krad showed a considerably better texture and were also given higher scores in the sensory tests. The Tables containing the distribution of the experimental results with the dial gauge demonstrate clearly the beneficial effect of high doses.

2.3.3. Evaluation of the results of titratable acidity tests. Investigation of the changes in titratable acidity has resulted in the finding of a relatively slight decrease in acidity after 10 days of storage (Table 20).

2.3.4. Evaluation of the results of the tests concerning changes in reducing sugar content. According to the data in Table 21 there is during storage a slight increase in the reducing sugar content of both the control and irradiated samples.

Table 20

Changes in the total acidity of Pink variety rambutan

Period of storage, days	ml of 0.1 N NaOH per 100 g of fruit			
	control	20 krad	60 krad	100 krad
3	26.60	17.40	21.00	19.75
7	32.80	(23.80)	32.60	21.70
10	25.80	21.00	22.00	18.60
18	—	10.00	13.55	—

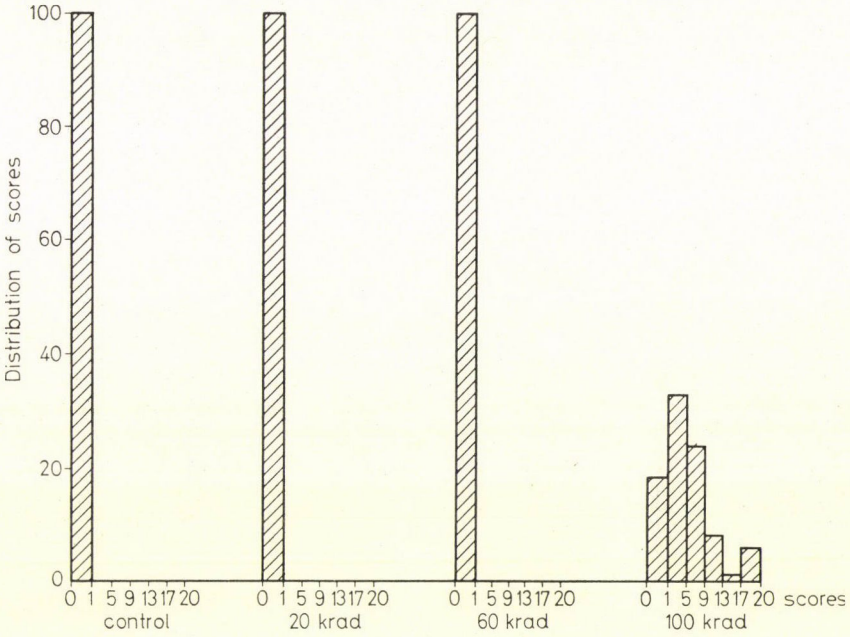

Fig. 7. The texture of rambutan after storage at 18 °C for 13 days

Table 21

Reducing sugar content of the two varieties of rambutan

Period of storage, days	Reducing sugar in mg of dextrose per g of rambutan							
	Pink variety						Bangyeekan variety	
	control	10 krad	20 krad	40 krad	60 krad	100 krad	control	40 krad
2	34.9	—	—	40.45	55.5	—	44.95	46.95
	38.74	—	40.17	—	41.52	40.87	—	—
8	34.7	38.2	—	—	—	—	—	—
	41.05	—	—	41.42	41.42	—	44.9	47.74
6	41.42	—	41.56	—	40.48	39.84	—	—
9	43.06	—	47.59	—	45.16	44.19	—	—
10	35.4	—	—	38 7	—	—	45.7	45.5

2.3.5. Evaluation of the results of sensory tests. Though there was no significant difference between the experimental data of the control and irradiated samples stored for three days (Table 22), the individual score sheets indicated better organoleptic properties in the case of samples irradiated with high doses than those found in the controls.

Table 22

Comparison of the organoleptic properties of control and radiation treated Pink variety rambutan

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	84	51		
Treatments	1	3	0.33	0.31
Persons	8	5	1.6	1.49
Interaction	45	15	3.0	2.8
Residue	30	28	1.07	

Period of storage: 3 days
Storage temperature: 18 °C

1st sample: control	$\overline{X}_1 = 5.64$
2nd sample: 20 krad	$\overline{X}_2 = 6.0$
3rd sample: 60 krad	$\overline{X}_3 = 6.75$
4th sample: 100 krad	$\overline{X}_4 = 6.15$

A significant difference was found when controls were compared to samples of the Bangyeekan and Pink varieties treated with 40 krad (Table 23).

Table 23

Comparison of the organoleptic properties of Pink and Bangyeekan variety rambutans

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	169	96		
Treatments	70	3	23.3	129
Persons	57	9	6.35	35.2
Interaction	32	27	1.19	6.6
Residue	10	57	0.18	

Period storage: 6 days
Storage temperature: 18 °C

1st sample: control (Pink variety)	$\overline{X}_1 = 6.78$
2nd sample: control (Bangyeekan variety)	$\overline{X}_2 = 6.00$
3rd sample: 40 krad (Pink variety)	$\overline{X}_3 = 5.85$
4th sample: 40 krad (Bangyeekan variety)	$\overline{X}_4 = 5.67$

The Pink variety was unequivocally better with a relatively great difference only in the scores given by various judges, as manifest in the high value of interaction.

Sensory tests carried out with Pink variety rambutan on the 9th day of storage gave unequivocally higher scores (Table 24) to fruit treated with higher doses. In general, the judges gave the highest scores to samples which had been treated with 100 krad.

Table 24

Comparison of the organoleptic properties of control and radiation treated Pink variety rambutan

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	93	59		
Treatments	32	3	10.7	15.80
Persons	4	6	0.67	0.98
Interaction	35	18	1.94	2.86
Residue	22	32	0.69	

Period of storage: 9 days
Storage temperature: 18 °C

1st sample: control $\overline{X}_1 = 5.68$
2nd sample: 40 krad $\overline{X}_2 = 5.71$
3rd sample: 60 krad $\overline{X}_3 = 5.37$
4th sample: 100 krad $\overline{X}_4 = 6.00$

Table 25

Changes in the vitamin C content of Pink variety rambutan

Storage, days	Dose, krad								Note: series of experiments
	control	10	20	30	40	50	60	100	
1	49.9	—	—	—	55.0	—	—	47.5	2nd
2	51.0	—	—	—	46.0	—	41.0	—	2nd
2	—	—	—	—	46.3	—	—	47.4	2nd
2	51.5	—	57.0	—	—	—	60.0	53.0	3rd
4	33.3	—	—	—	38.3	—	—	38.9	1st
4	33.3	—	—	—	38.9	—	—	40.0	1st
6	54.0	—	58.7	—	—	—	57.6	53.0	3rd
7	37.2	—	—	—	43.9	—	—	50.4	2nd
9	60.1	—	59.5	—	—	—	63.5	59.4	3rd
11	51.1	47.8	48.3	34.9	41.0	39.7	45.6	—	1st

Table 26

Changes in the vitamin C content of Bangyeekan variety rambutan

Storage days	Control	Dose, krad		Note
		40	100	
2	23.4	28.3	23.4	2nd series
4	20.0	23.3	22.2	1st series
11	21.3	24.6	—	1st series

2.3.6. Evaluation of the results of vitamin C determinations. Determination of the vitamin C content has led to the finding that the Pink variety contains considerably more vitamin C than the Bangyeekan variety rambutan, but changes in vitamin C content due to irradiation were slighter in the Bangyeekan variety than in the Pink variety (Tables 25 and 26). Testing of whole, halved and peeled rambutan samples has shown that after irradiation the vitamin C content of the peeled fruits is lower. The experimental results of vitamin C content determinations have in general indicated the absence of any significant decrease in vitamin C content of rambutan samples exposed to various radiation doses.

2.4. Evaluation of longan tests

2.4.1. Evaluation of the results of storage experiments. As data on the storage of longan were not available in the literature prior to the beginning of the storage experiments preliminary tests were carried out with samples irradiated at 10, 50 and 100 krad, respectively. It appeared from the data of these preliminary experiments that in the case of longan higher doses have a beneficial effect on the changes in the texture of the fruit. Later tests carried out after 15 to 20 days storage confirmed this finding.

In the following storage experiments higher doses of 100, 150, 200 and 250 krad were applied. In the case of high doses the weight loss was considerably lower when compared to the control (Table 29).

The daily tests performed for the determination of the rotting index revealed that after 16 days storage the rotting index of both the control and of the sample irradiated with 10 krad was 100%. Investigation of the effect of higher doses has shown that up to 60 krad the beneficial effect of irradiation cannot be demonstrated, moreover at 30 krad a marked softening of the flesh of the fruit was observed. It was found that when samples were irradiated with high doses of 150 and 200 krad, both the rotting index and the texture of the fruits showed after 35 days of storage a condition similar to that of the control fruits after 16 days (Table 27).

Table 27

Rotting indices of control and radiation treated longan

Storage, days	Control	Dose, krad						
		10	30	60	80	100	150	200
1	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	68	54	54	10	18	26	20	0
15	98	98	(90)	92	38	53	30	0
16	100	100	46	94	46	(94)	30	10
17	100	100	90	(98)	46	72	36	16
21	100	100	82	94	66	80	40	20
22	—	—	100	(90)	88	(100)	(68)	—
23	—	—	90	(90)	90	84	(58)	40
24	—	—	96	(90)	90	90	34	42
27	—	—	100	94	—	100	(80)	—
29	—	—	—	100	100	100	86	16
30	—	—	—	—	(88)	—	(40)	20
31	—	—	—	—	100	—	80	30
34	—	—	—	—	—	—	80	68
35	—	—	—	—	—	—	100	—
36	—	—	—	—	—	—	100	90

Table 28 contains the results obtained with doses having a beneficial effect. The data show that in cases of 200 and 250 krad doses almost no deterioration of quality has occurred in the first two weeks of storage. It appears further from these data that the rotting of longan, like that of rambutan, proceeds at a higher rate only after an initial period. From the aspect of rotting the fruit lot did not appear to be homogeneous, particularly at higher doses. It was, however, found that the rotting of samples irradiated with higher doses is more protracted, and the difference in the time of rotting of the individual samples increases more and more. This appears clearly from Table 30b in which a summary of the results is given.

It appears from the weight loss data of stored longan (Table 29) that higher doses have a definitely beneficial effect on the state of the fruit. In a diagrammatic representation (Fig. 8) it seems that the loss of water might be divided into 3 phases:

a) In the first 3 days it reaches 6% independently of the dose applied.

b) Till the 16th—18th day the weight loss of samples irradiated with low doses (up to 150 krad) is 0.75% per day, when higher doses are applied this drops practically by half.

Table 28

Rotting indices of control and radiation treated longan

Storage, days	Control	Dose, krad				
		50	100	150	200	250
1	0	0	0	0	0	0
2	0	0	0	0	0	0
5	0	0	0	0	0	0
8	30	32	6	4	0	0
10	70	32	8	4	8	0
12	98	60	16	4	8	8
15	100	90	80	36	20	20
17	—	100	80	36	20	28
19	—	—	88	50	—	—
22	—	—	93	66	—	—
24	—	—	100	70	42	28

c) From the 18th day onward the rate of weight loss increases, but it is not possible to extrapolate satisfactorily the final weight loss from the period of the test.

Table 29

Weight loss of longan during storage at 18 °C (%)

Storage, days	Control	Dose, krad				
		50	100	150	200	250
1	0	0	0	0	0	0
2	6.3	6.4	6.2	6.1	5.7	6.2
3	7.4	7.6	8.3	8.1	6.7	6.2
4	8.5	8.7	8.3	8.1	6.7	6.2
5	9.5	9.8	10.4	10.2	9.6	10.4
10	12.7	12.0	12.5	12.2	9.6	10.4
11	12.7	13.1	12.5	12.2	10.6	10.4
12	13.8	13.1	12.5	12.2	10.6	10.4
15	17.02	16.4	15.6	15.3	11.6	10.4
16	19.1	17.5	17.7	1.63	12.6	13.5
17	21.2	19.7	17.7	17.3	13.5	14.5
18	21.2	20.9	18.7	17.3	16.5	14.5
19	23.4	23.1	20.8	17.3	16.5	14.5
22	30.8	32.9	29.1	22.4	21.3	14.5
23	36.1	38.4	32.2	24.4	23.3	15.6
24	37.2	40.6	35.4	26.5	23.3	16.6

The maximum curvature of the curves representing the weight loss is between the 18th and 22nd day (Fig. 8). On the 20th day the effect of the dose is quite clearly apparent. While up to 50 krad irradiation had no significant effect, above 50 krad there was a linear drop in weight loss. The data are summarized in Table 30/a.

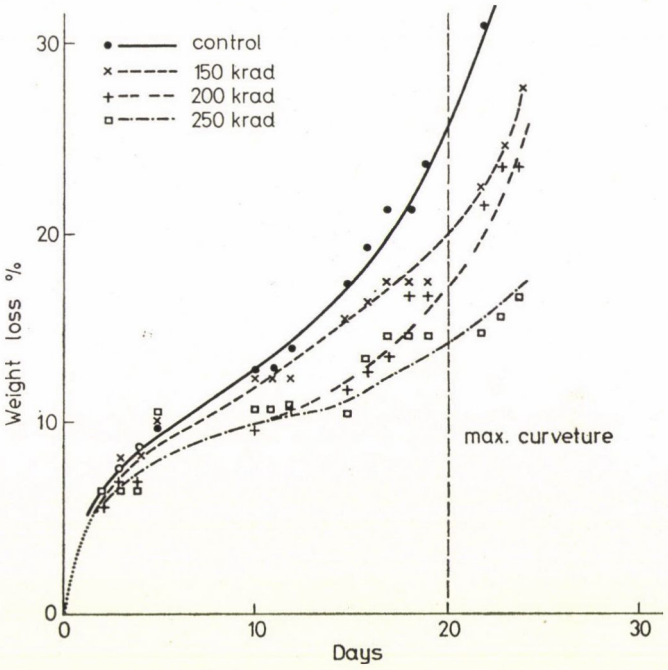

Fig. 8. The weight loss of longan vs. the period of storage

The average rotting time and the standard deviaton of the latter were investigated as functions of the dose from the rotting indices obtained in the storage tests (Table 30/b). When the average rotting time is plotted vs. weight loss (Fig. 9) it appears that the effect of irradiation considerably improves storability according to a curve of second order.

Table 30/a

Weight loss of irradiated longan

Dose, krad	0	50	100	150	200	250
Weight loss, %	26	26	22	19.5	17.5	14

The variance of rotting time is approximately proportional to the average rotting time (Fig. 10).

A likely explanation for this might perhaps be found in the correlation between the rotting and radiation sensitivity of the fruit specimens. Rotting of the rapidly deteriorating specimens is less influenced by irradiation than that of specimens which are less liable to rotting.

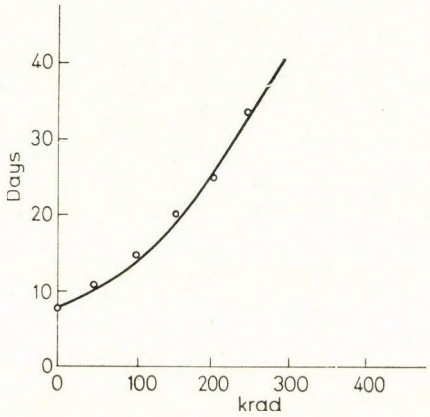

Fig. 9. Average rotting period of longan vs. radiation dose

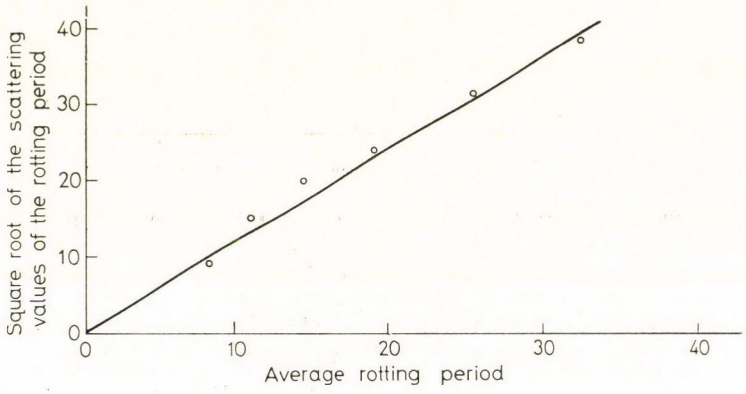

Fig. 10. Correlation between the average rotting period of longan and the standard deviation of the results

Table 30/b

Average rotting period of longan and the standard deviation of the results

Dose, krad	0	50	100	150	200	250
Average rotting time, days	8.2	10.6	14.7	19.4	25.5	33
Standard deviation	0.8	2.9	3.9	5.2	9.5	14.3

2.4.2. Evaluation of the results of texture tests. The texture of each fruit in lots of 10 was examined by means of 5 punctures and it was found that irradiation had a beneficial effect on texture (Tables 31 and 32). Figures 11 and 12 show the distribution of the results of texture tests on the 9th and 16th day of storage.

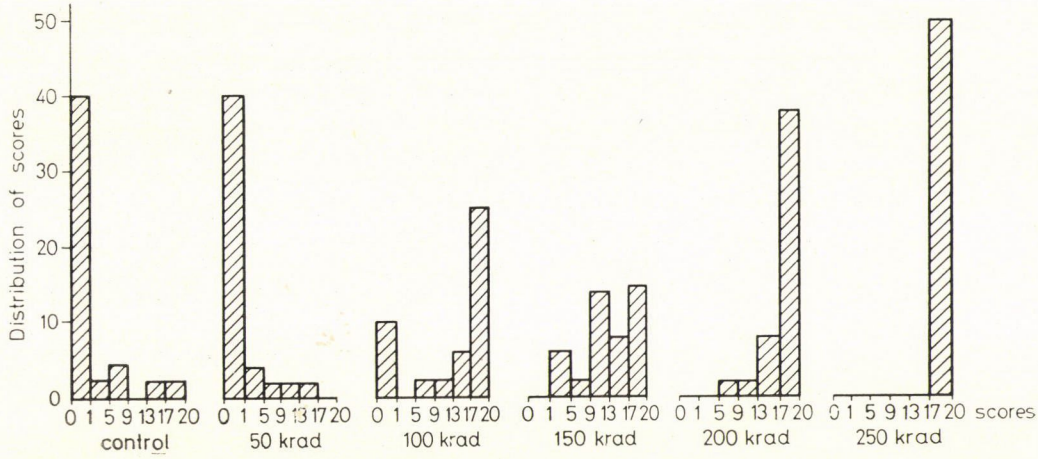

Fig. 11. The texture of longan after 9 days storage at 18 °C

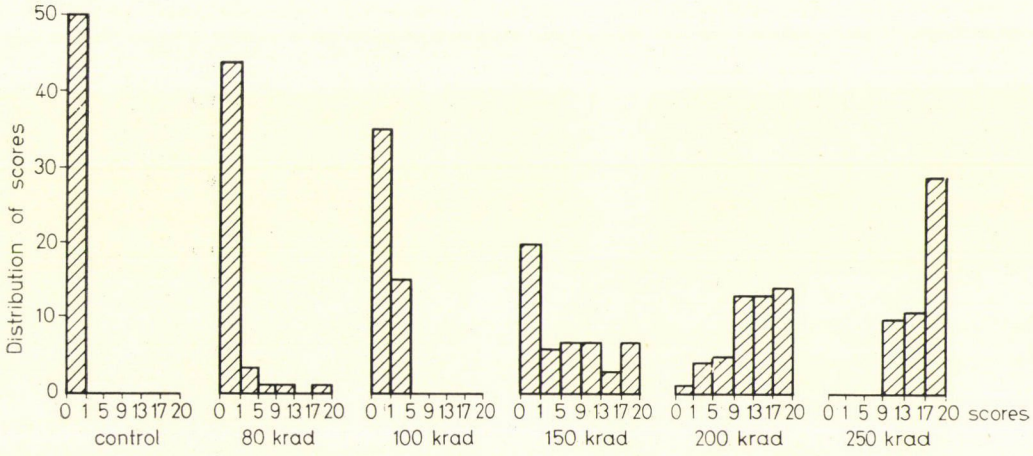

Fig. 12. The texture of longan after 16 days storage at 18 °C

It appears from the Figures that texture tests during storage have given in case of higher doses values representing higher resistance.

2.4.3. Evaluation of the results of sensory tests. After 3 days storage the sensory test scores of the control and irradiated longan samples failed to reveal any significant difference, though the judges gave higher scores generally to the irradiated samples than to the controls (Table 33).

Table 31

Texture of longan vs. period of storage

Storage, days	Treatment	Scale division (dial gauge)		
		$\bar{X}$	minimum	maximum
2	control	16.53	4.1	20.0
	30 krad	17.82	6.5	20.0
	60 krad	15.78	5.3	20.0
	80 krad	16.11	6.3	20.0
	100 krad	13.16	3.4	200.
	200 krad	16.35	4.5	20.0
7	control	7.2	0.0	20.0
	10 krad	8.88	0.0	20.0
	30 krad	7.76	0.0	20.0
	60 krad	15.07	0.1	20.0
	100 krad	10.0	0.0	20.0
	150 krad	11.54	1.3	200.
9	control (18 °C)	3.72	0.0	20.0
	control (23 °C)	7.58	0.0	20.0
	control (16 °C)	13.32	2.4	20.0
	10 krad	1.54	0.0	12.9
	80 krad	7.66	0.0	20.0
	150 krad	8.78	0.0	20.0
	200 krad	11.4	0.0	20.0
16	80 krad	0.95	0.0	20.0
	100 krad	0.84	0.0	3.7
	150 krad	6.54	0.4	20.0
	200 krad	11.15	0.5	20.0

When the controls and irradiated samples were compared by organoleptic tests on the 8th day, statistical evaluation revealed a significant difference between the effects of the treatments (Table 34). Evaluation has, however, involved a relatively high interaction between the judges and the treatments in the course of ranking. It must be also added that because of the relatively low amount of volatiles the judges were faced with a rather difficult task when ranking longan, since they had to decide on slight sensory differences. This series of experiments was complemented by sensory tests carried out on the 9th day; the data of this latter test are shown in Table 35 and appear to be similar to those in the foregoing Table.

Table 32

Texture of longan vs. period of storage

Storage, days	Treatment	Scale division (dial gauge)		
		$\bar{X}$	minimum	maximum
2	control	17.83	1.8	18.68
	50 krad	17.05	6.8	20.00
	100 krad	16.43	2.3	20.00
	150 krad	16.73	3.3	20.00
	200 krad	15.43	3.7	20.00
	250 krad	17.66	9.5	200.0
9	control	1.03	0.0	18.6
	50 krad	0.79	0.0	14.6
	100 krad	12.78	6.6	20.00
	150 krad	13.38	2.7	20.00
	200 krad	12.61	0.0	20.00
	250 krad	19.65	17.3	20 00
17	control	0.0	0.0	0.0
	50 krad	0.0	0.0	0.0
	100 krad	1.37	0.0	20.0
	150 krad	1.69	0.0	20.0
	200 krad	1.62	0.0	20.0
	250 krad	16.79	9.2	20.0

Table 33

Comparison of the organoleptic properties of control and radiation treated longan after 3 days storage

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	15	79		
Treatments	2	3	0.65	0.34
Persons	2	9	0.22	0.12
Interaction	35	27	1.29	0.68
Residue	76	40	1.90	

Period of storage: 3 days
Storage temperature: 18 °C

1st sample: control $\bar{X}_1 = 6.55$
2nd sample: 30 krad $\bar{X}_2 = 6.65$
3rd sample: 60 krad $\bar{X}_3 = 6.90$
4th sample: 100 krad $\bar{X}_4 = 6.65$

Table 34

Comparison of the organoleptic properties of control and radiation treated longan after 8 days storage

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	365	99		
Treatments	22	4	5.5	5.10
Persons	19	9	2.11	1.99
Interaction	271	36	7.52	7.1
Residue	53	40	1.06	

Period of storage: 8 days
Storage temperature: 18 °C

1st sample: control $\bar{X}_1 = 6.0$
2nd sample: 10 krad $\bar{X}_2 = 6.4$
3rd sample: 30 krad $\bar{X}_3 = 6.65$
4th sample: 80 krad $\bar{X}_4 = 5.75$
5th sample: 200 krad $\bar{X}_5 = 6.95$

It has to be stressed as one of the most important results of the sensory evaluation of longans, both of the controls and of fruits irradiated with high doses, that the judges agreed in a total absence of off-flavour due to irradiation.

2.4.4. Evaluation of the results of titratable acidity. It appears from the results summed up in Table 36 that there is but a very slight change in titratable acidity as a function of storage time. Similarly to the other tropical

Table 35

Comparison of the organoleptic properties of control and radiation treated longan

Components of standard deviation	SSQ	DF	S^2	Significance level
Total	240	71		
Treatments	37	3	12.3	15.2
Persons	1	8	0.125	0.154
Interaction	173	24	7.21	8.9
Residue	29	36	0.81	

Period of storage: 9 days
Storage temperature: 18 °C

1st sample: control $\bar{X}_1 = 6.88$
2nd sample: 100 krad $\bar{X}_2 = 6.77$
3rd sample: 150 krad $\bar{X}_3 = 6.88$
4th sample: 200 krad $\bar{X}_4 = 6.77$

Table 36

Acidity changes of longan

Storage, days	0,1 N NaOH, ml per g of fruit					
	control	10 krad	30 krad	50 krad	60 krad	100 krad
8	(14.4)	17.0	18.6	—	18.4	17.2

fruits investigated by us, in the case of longan too no unequivocal relationship was found between the data of changes as induced by irradiation.

2.4.5. Evaluation of the results of reducing sugar determinations. In connection with the investigation of the changes in reducing sugar content it should be stressed that a slight increase compared to the initial value was found only in samples irradiated with higher doses (200 to 250 krad) and that only after a longer storage period (Table 37).

According to the data of the reducing sugar determinations performed at the beginning of the storage tests a slight decrease occurs in case of higher doses. This is the direct consequence of the radiation effect, since this difference is eliminated in a few days.

2.4.6. Evaluation of the results of vitamin C determinations. At the beginning of the storage period a slight difference was observed in the vitamin C contents of the irradiated and untreated control longan samples (Table 38). However, after a longer storage period, in agreement with the literary data on other fruits, no difference was detected between the controls and the irradiated sampled.

Table 37

Reducing sugar content of longan

Storage, days	Control	Reducing sugar in mg of dextrose									Note: series
		10	30	50	60	70	100	150	200	250	
		krad									
1	57.4	—	—	56.9	—	—	54.1	52.7	54.1	—	2nd
3	63.2	—	61.2	—	59.5	61.0	—	—	—	—	1st
8	55.3	56.9	56.6	—	55.8	—	52.9	59.5	—	—	1st
10	60.5	61.9	—	—	—	59.2	—	60.9	62.8	—	1st
17	—	—	—	—	—	74.5	68.0	61.1	66.8	(85.2)	1st (2nd)
21	—	—	—	—	—	—	—	77.7	82.6	84.0	2nd
21	—	—	—	—	—	—	—	72.9	77.3	81.9	2nd

Table 38

Changes in the vitamin C content of longan during storage at 18 °C

Storage, days	Control	Dose, krad							Note: series
		30	50	60	80	100	150	200	
2	72.5	72	—	73.3	64.8	(73.0)	—	65.5	1st
7	74.3	81.0	—	83.3	—	78.0	—	68.3	1st
9	83.0	—	—	—	72.0	—	(67.0)	79.5	1st
16	—	—	—	—	110.0	96.2	93.0	95.4	1st

After the rotting of the controls the still intact samples of fruit irradiated with 100, 150 and 200 krad, respectively, gave particularly favourable results.

*

Finally, the authors wish to thank Dr. Svasti Srisukh, Head of the Office of Atomic Energy for Peace (Bangkok) for his immediate help in the performance of their programme, as well as the team of the Biology Department for their conscientious and enthusiastic cooperation without which successful work would have been impossible.

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PRODUCTION OF PECTOLYTIC ENZYMES BY ASPERGILLI IN SUBMERGED CULTURE

PART I – EFFECT OF THE NITROGEN SOURCE ON THE PRODUCTION OF POLYGALACTURONASE

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(Received January 20, 1971)

The effect of various nitrogen sources on the polygalacturonase (PG) production of *Aspergillus awamori* was examined.

Yeast extract and corn-steep liquor were used as complex organic nitrogen sources. The optimal levels of added inorganic and organic nitrogen sources were determined.

Of the nitrogen sources tested [$(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, NH_4NO_3, $NaNO_3$, $Ca(NO_3)_2$ and $NH_2 \cdot CO \cdot NH_2$], $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ proved to be the best for the synthesis of polygalacturonase by this fungus. (PG concentration of the culture fluid: $SPA_{75}^{Obi} = 3142$ and 2780 l/h/l resp.)

The simultaneous use of 20 g $(NH_4)_2SO_4$, 20 g corn-steep liquor and 50 g yeast extract in 1 litre of the media proved to be most advantageous for the production of polygalacturonase by *Aspergillus awamori*. With each component, decrease in concentration resulted in a lower yield of polygalacturonase. Further increase in the concentration of yeast extract and corn-steep liquor (to 100 ml/l and 50 g/l, resp.) increased polygalacturonase activity exclusively at lower levels of $(NH_4)_2SO_4$(6 and 10 g/l), the effect being the opposite at a concentration of 20 g/l $(NH_4)_2SO_4$.

Inorganic nitrogen compounds like ammonium salts and nitrates are usually appropriate sources of nitrogen for most fungi. Microorganisms utilize nitrate in one of the two following ways: 1. assimilation into cell nitrogen, or 2. dissimilation or nitrate respiration, where nitrate acts as an alternative hydrogen acceptor to oxygen (NICHOLAS, 1965).

Some microorganisms prefer nitrate to ammonia (ZETELAKI, 1966). WALKER and NICHOLAS (1961) reported that some fungi grown with limited air supply dissimilated nitrate rapidly as an alternative hydrogen acceptor to oxygen, resulting very often in accumulation of toxic amounts of free nitrite in the medium.

Nitrate assimilation can be suppressed by ammonia in fungi. If *Scopulariopsis brevicaulis* is grown first in nitrate and ammonium sulphate is then added on the fourth day, the assimilation of nitrate is stopped by the immediate assimilation of ammonia, while ammonia assimilation can not be influenced by the addition of nitrate (MORTON & MACMILLAN, 1954).

Sources of accessory factors are often needed in microbiological practice. When present in the culture media, these factors stimulate the growth of microorganisms and often give increased yields of the desired metabolic prod-

uct. These factors are available in corn-steep liquor and also in yeast extract, which are commonly used as complex organic nitrogen sources for the cultivation of microorganisms (SMITH, 1960).

Nitrogen demand varies with the species of microorganism and with the method of production as well.

The present paper deals with experiments carried out to determine the effect of different nitrogen sources on the growth and polygalacturonase synthesis of *Aspergillus awamori*.

1. Materials and methods

1.1. Microorganism

An UV variant (No. 273) of *Aspergillus awamori* produced in this laboratory was used as the test organism. (ZETELAKI-HORVÁTH & DOBRA-SERES, 1972).

1.2. Inoculation

10% of 24-hour vegetative cultures were used.

1.3. Composition of media

The basal medium for testing nitrogen sources was as follows:

Sucrose: 100 g, KH_2PO_4: 2 g, corn-steep liquor: 10 g/litre.

Nitrogen sources, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, NH_4NO_3, $NaNO_3$, $Ca(NO_3)_2$ and $NH_2 \cdot CO \cdot NH_2$, resp., were added to the media at a level of 2.1 g N/litre.

For the investigation of the effect of nitrogen, 27 kinds of media were prepared, in which concentrations of sucrose (100 g/l) and KH_2PO_4 (2 g/l) were kept constant, while the concentrations of $(NH_4)_2SO_4$ (6, 10 and 20 g/l), yeast extract (20, 50 and 100 ml/l) and corn-steep liquor (10, 20 and 50 g/l) were varied.

The effect of each recipe on the production of polygalacturonase of *Aspergillus awamori* was tested in three parallel shake culture experiments using 100 ml of medium in 500 ml Erlenmeyer flasks.

Yeast extract: 200 g of bakers' yeast were mixed with 1 litre of water and autoclaved at a pressure of 2 atm for 15 minutes. The cell debris were separated by centrifugation. The volume of the supernatant was made up to 1 litre and this yeast extract was added to the media at various concentration levels. Corn-steep liquor (containing 51% dry matter) was added to the media at various concentration levels.

1.4. Cultural conditions

Cultures were incubated in 500-ml Erlenmeyer flasks, containing 100 ml of medium, for 72 hours, on a shaking machine of 330 rpm with a stroke length of 20 mm. Oxygen solution rate in the flasks was 17 mmole O_2/l/h, as determined by the sulphite oxidation method of COOPER et al. (1944). The temperature during cultivation was 28 °C.

1.5. Mycelial weight

Mycelial weight was determined after drying the mycelia at 105 °C to constant weight.

1.6. Evaluation of polygalacturonase concentration

Activity of polygalacturonase was measured by viscosimetry. Changes in the viscosity were measured on an 0.25 % apple pectin solution (Obipektin, type: Violettband, Obipektin A. G., Bischofszell, Schweiz, the degree of esterification was 33 %) as the substrate. Fall in viscosity of the substrate solution was measured and enzyme concentration expressed as SPA_{75}^{Obi}* (Specific Pectolytic Activity, 1/h/l). SPA_{75} is the quantity (litre) of substrate solution decomposed to reach a loss of 75 % of its original specific viscosity by 1 litre of the enzyme solution (culture filtrate) during one hour of incubation at 50 °C (VAS, 1953).

2. Results

2.1. The effect of well-defined nitrogen sources

The effects of various nitrogen sources on growth and polygalacturonase production of *Aspergillus awamori* were examined in shake culture. The results of the experiments are given in Fig. 1. The curves represent the mean values of four experiments.

As can be seen in Fig. 1 mycelial yield of the cultures grown in media containing different nitrogen sources was high enough with all nitrogen sources. The highest yield was obtained when $(NH_4)_2SO_4$ (3.5 %), $(NH_4)_2HPO_4$ and $NaNO_3$ (3.3 %), resp., were used as the nitrogen source. The lowest mycelial yield (2.27 %) was obtained in the media containing $Ca(NO_3)_2$. Addition of urea or ammonium nitrate as the nitrogen source to the media of *Aspergillus awamori* resulted in a mycelial yield of 2.9 and 2.7 per cent, respectively.

*(The upper index Obi refers to the nature of the substrate.)

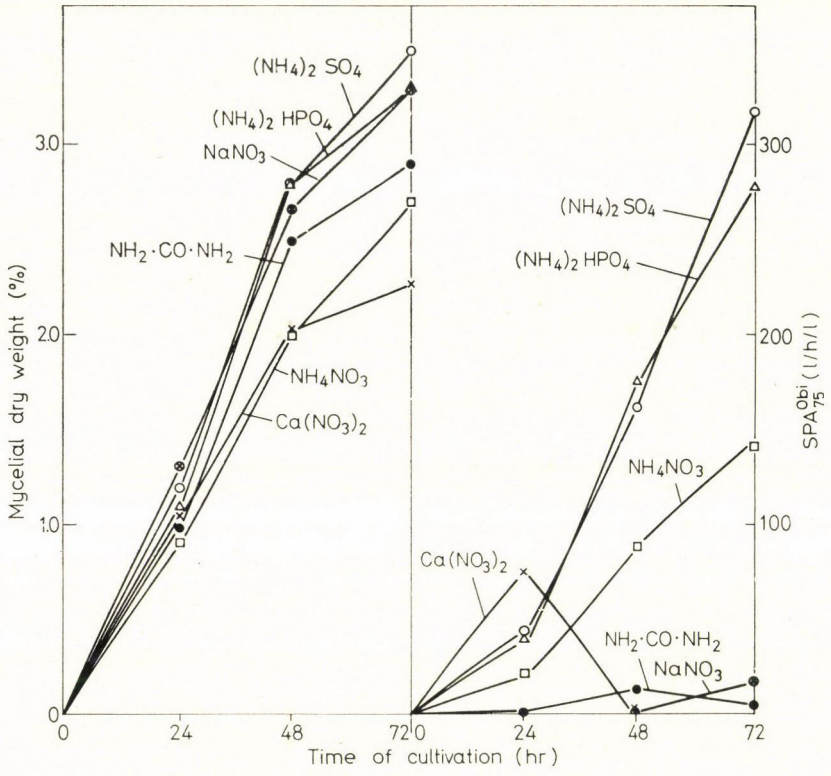

Fig. 1. Growth and polygalacturonase production of *Aspergillus awamori* in shake culture in media containing different nitrogen sources

Varying the nitrogen source of the media caused significantly greater changes in the polygalacturonase yield than in the mycelial yield of the culture. As shown in Fig. 1, the highest polygalacturonase concentrations (3175 and 2780 SPA_{75}^{Obi} 1/h/l) were measured in the cultures grown in media containing $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$, respectively. A much lower concentration of polygalacturonase (SPA_{75}^{Obi} = 1409 1/h/l) was measured when NH_4NO_3 was used in the medium. The lowest polygalacturonase concentrations (SPA_{75}^{Obi} = = 200) were obtained when NH_4NO_3 and $NH_2 \cdot CO \cdot NH_2$ were applied as the nitrogen source. There was some polygalacturonase activity detectable in the 24-hour culture grown with $Ca(NO_3)_2$ as the nitrogen source, but on the 2nd and 3rd day of cultivation, polygalacturonase was not measurable due to the gel-formation caused by the presence of calcium.

Growth and polygalacturonase production of *Aspergillus awamori* cultures in media of different nitrogen sources are shown in Figs. 2 and 3.

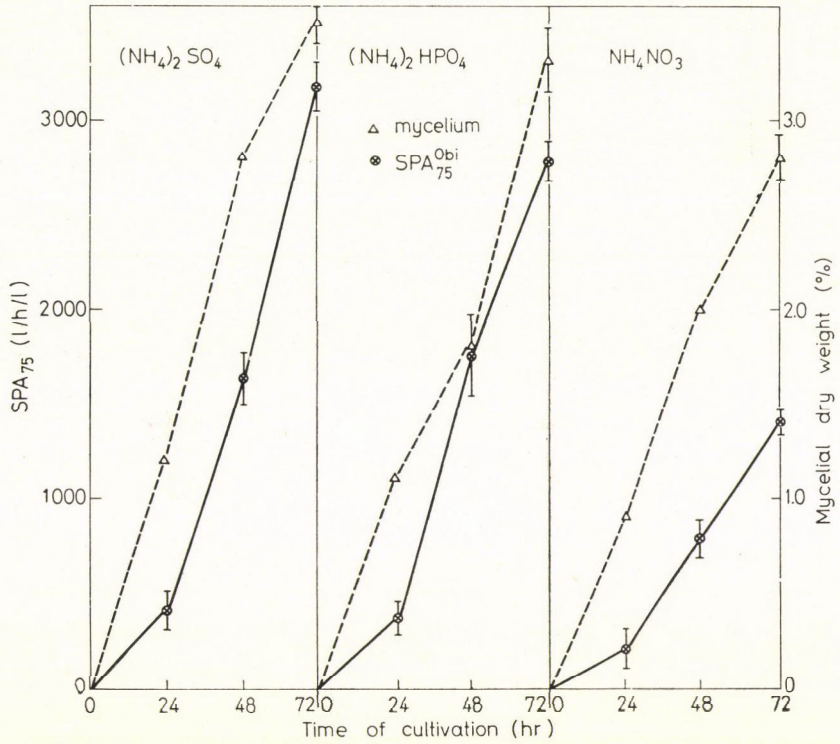

Fig. 2. Growth and polygalacturonase production of *Aspergillus awamori* in shake culture, with $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$ and NH_4NO_3, resp., used as the nitrogen source (with standard deviation indicated)

2.2. The effect of complex nitrogen sources

In the present work yeast extract, rich in vitamins and amino acids and corn-steep liquor were added to the media of *Aspergillus awamori* cultures.

Production of polygalacturonase by *Aspergillus awamori* at different concentrations of yeast extract and corn-steep liquor, with $(NH_4)_2SO_4$ added at concentrations of 6.10 and 20 g/l, resp., can be seen in Figs 4, 5 and 6.

Apparently, in media containing $(NH_4)_2SO_4$, the addition of 50 ml/l yeast extract and 50 g/l corn-steep liquor was most advantageous for the production of polygalacturonase by the mould (Fig. 4).

Increasing the $(NH_4)_2SO_4$ concentration from 6 to 10 g/l always resulted in a higher yield of polygalacturonase (Fig. 5). The highest polygalacturonase concentration was obtained by addition of 50 ml/l yeast extract and 20 g/l corn-steep liquor.

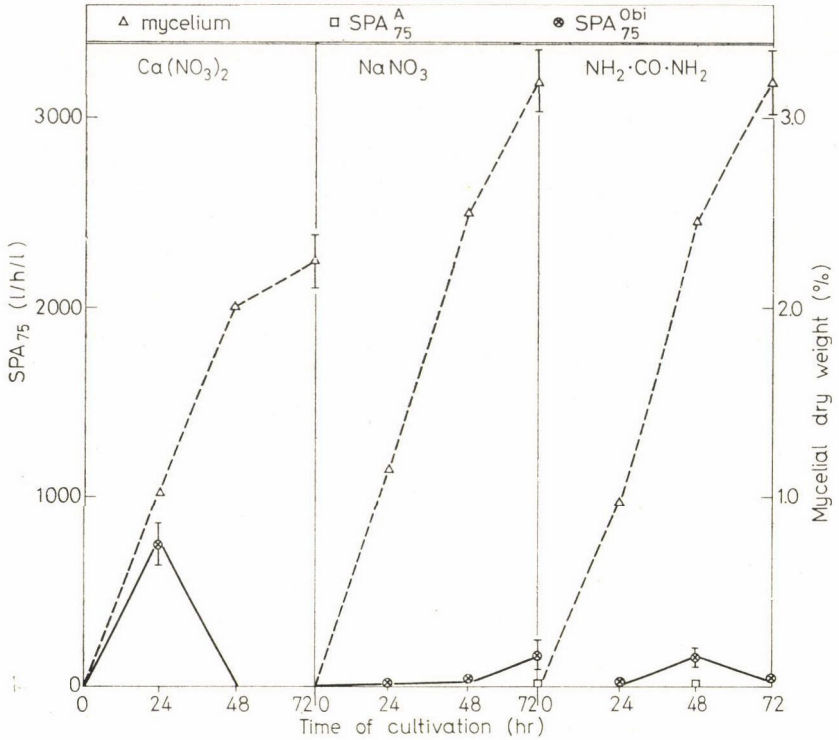

Fig. 3. Growth and polygalacturonase production of *Aspergillus awamori* in shake culture, with $Ca(NO_3)_2$, $NaNO_3$ and $NH_2 \cdot CO \cdot NH_2$, resp., used as the nitrogen source (with standard deviations indicated)

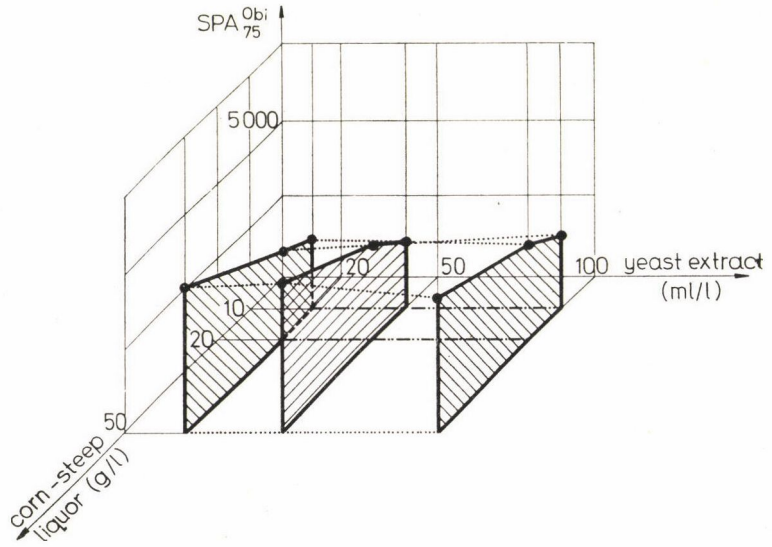

Fig. 4. Polygalacturonase production of the 72-hour shake culture of *Aspergillus awamori* as a function of the concentration of corn-steep liquor and yeast extract in the medium with $(NH_4)_2SO_4$ added at a concentration of 6 g/l

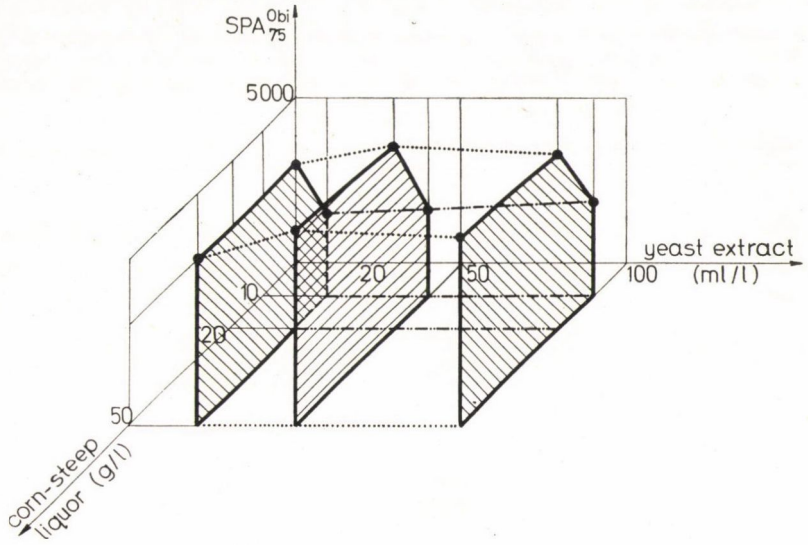

Fig. 5. Polygalacturonase production of the 72-hour shake culture of *Aspergillus awamori* as a function of the concentration of corn-steep liquor and yeast extract in the medium with $(NH_4)_2SO_4$ added at a concentration of 10 g/l

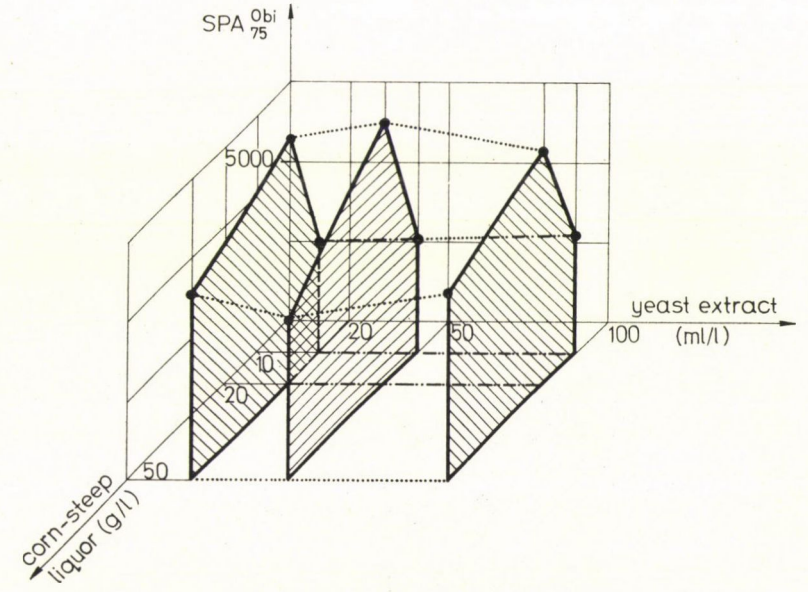

Fig. 6. Polygalacturonase production of the 72-hour shake culture of *Aspergillus awamori* as a function of the concentration of corn-steep liquor and yeast extract in the medium with $(NH_4)_2SO_4$ added at a concentration of 20 g/l

A further increase in the concentration of $(NH_4)_2SO_4$ to 20 g/l resulted in the highest yield of polygalacturonase with 50 g/l yeast extract and 20 g/l corn-steep liquor added to the basal medium (Fig. 6).

Production of polygalacturonase by *Aspergillus awamori* cultures grown in media containing 100 g/l sucrose, 20 g/l KH_2PO_4 and 50 ml/l yeast extract, is shown as a function of the concentration of $(NH_4)_2SO_4$ and corn-steep liquor, respectively in Fig. 7.

Fig. 7 shows the addition of 20 g/l $(NH_4)_2SO_4$ and corn-steep liquor to have resulted in the highest production of polygalacturonase.

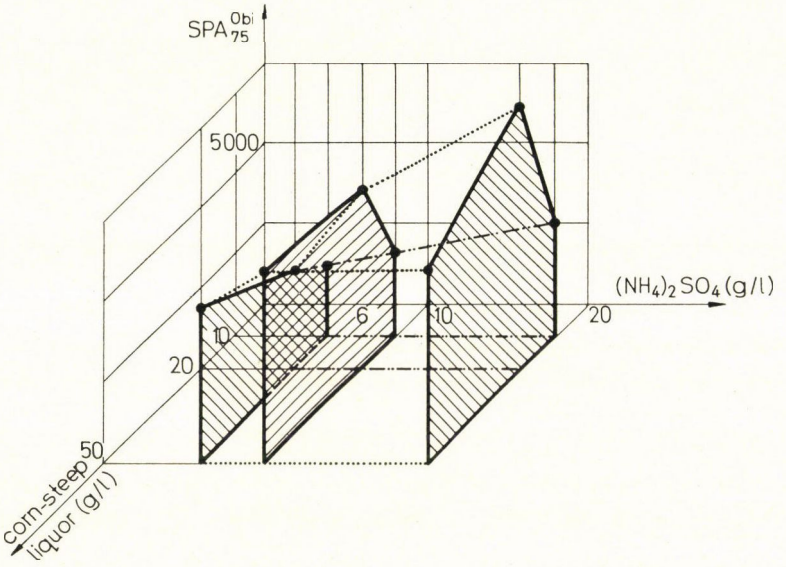

Fig. 7. Polygalacturonase production of the 72-hour shake culture of *Aspergillus awamori* as a function of the concentration of $(NH_4)_2SO_4$ and corn-steep liquor with yeast extract added at a concentration of 50 ml/l

3. Discussion

For the production of polygalacturonase from *Aspergillus awamori*, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, NH_4NO_3, $Ca(NO_3)_2$ and urea were used as nitrogen sources in the media.

The polygalacturonase production of this mould strain was significantly higher (at a probability level of 99.9 %) in the ammonium sulphate containing medium (SPA_{75}^{Obi} = 3142 l/h/l) than in those containing other nitrogen sources as specified above (Table 1).

$(NH_4)_2HPO_4$ proved to be the second best nitrogen source for the polygalacturonase production of *Aspergillus awamori* while VASU (1963), MALTSENKO and co-workers (1966) and TEJERINA (1969) described $(NH_4)_2HPO_4$ to be the best nitrogen source for polygalacturonase production by *Aspergillus niger* and *Erwinia carotovora*, respectively.

Table 1

Statistical analysis of polygalacturonase production by 72-hour cultures of Aspergillus awamori

Nitrogen content of culture fluid: 2.1 g/l

N-source	SPA_{75}^{Obi} (mean) $\bar{x}$ (l/h/l)	Standard deviation (s) (l/h/l)	Degrees of freedom (DF)	Comparison of the various N-sources with $(NH_4)_2SO_4$	
				t	P(%)
$(NH_4)_2SO_4$	3142	124	8	—	—
$(NH_4)_2HPO_4$	2780	52	8	5.24	>99.9
NH_4NO_3	1407	14	8	27.60	>99.9
$NaNO_3$	164	7	8	42.00	>99.9
$NH_2 \cdot CO \cdot NH_2$	45	5	8	47.74	>99.9

VASU (1963), using urea as nitrogen source, found a rather good polygalacturonase yield in *Aspergillus niger* culture while, according to our results, the production of polygalacturonase by *Aspergillus awamori* was negligible with this nitrogen source.

NH_4NO_3 was utilized as nitrogen source for pectolytic enzyme formation by *Aspergillus niger* (TUTTOBELLO & MILL, 1961), by *Bothrytis cinerea* (KAJI & TAGAWA, 1966) and by *Penicillium expansum* (SWINBURNE & CORDEN, 1969). $NaNO_3$ served as nitrogen source in production media of endopolygalacturonase from *Aspergillus saitoi* (YAMASAKI et al., 1966). We found a low polygalacturonase yield ($SPA_{75}^{bi} = 1407$ and 164 resp.) of *Aspergillus awamori* in the media containing NH_4NO_3 and $NaNO_3$, respectively.

TEJERINA and FERNANDEZ (1966) stated that a complex nitrogen source was required for the production of polygalacturonase from *Erwinia carotovora*. TEJERINA (1969) found the use of $(NH_4)_2HPO_4$ as the sole nitrogen source to be insufficient for the synthesis of polygalacturonase by this microorganism. Addition to the media of amino acids, namely of L-cystine was found to have the most pronounced stimulatory effect on enzyme synthesis. Though the presence of L-cystine is essential for the polygalacturonase synthesis of *Erwinia carotovora*, it represented the lowest proportion among the identified amino acid components of endopolygalacturonase of *Aspergillus niger* (REXOVÁ-BENKOVÁ & SLEZÁRIK, 1966).

Usually, amino acids are added to culture media not in the pure form, but as accessory factors of natural components (vitamins, amino acids). Yeast extract, containing 14 amino acids (JEREBZOFF, 1965), is often used in culture media of microorganisms for pectolytic enzyme formation (SMITH, 1960; ARIMA et al., 1964; BALKAY & VAS, 1968).

The effects of various concentrations of yeast extract, corn-steep liquor and ammonium sulphate on the polygalacturonase production by *Aspergillus*

awamori were examined. The addition of 20 g/l $(NH_4)_2SO_4$, 20 g/l corn-steep liquor and 50 ml/l yeast extract proved most advantageous for the polygalacturonase production of *Aspergillus awamori* (Table 2).

Table 2

Mean values ($\bar{x}$) and standard deviations (s) of polygalacturonase concentration in 72-hour Aspergillus awamori cultures

Number of parallel fermentations N = 4

Components added (g/l)		$(NH_4)_2SO_4$ (g/l)					
		6		10		20	
Yeast extract	Corn-steep liquor	SPA^{Obi}_{75} (l/h/l)		SPA^{Obi}_{75} (l/h/l)		SPA^{Obi}_{75} (l/h/l)	
		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
20	10	2164	725	2493	691	3461	160
20	20	2767	722	5003	1568	7756	2195
20	50	4642	1102	5178	1438	5086	369
50	10	2137	630	2673	383	3547	479
50	20	3059	677	5593	2121	8181	1368
50	50	4859	663	5926	702	6100	604
100	10	2355	510	2813	111	3711	223
100	20	3064	709	5328	933	7318	1023
100	50	4375	1277	5724	1161	5845	1199

This is in contrast to the results of TUTTOBELLO and MILL (1961) who found corn-steep liquor to be inhibitory for the pectolytic enzyme formation of *Aspergillus niger*. YAMASAKI and co-workers (1966) also reported that elevated concentration of corn-steep liquor in the media resulted in decreased production of endopolygalacturonase by *Aspergillus saitoi*.

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PRODUCTION OF PECTOLYTIC ENZYMES BY ASPERGILLI IN SUBMERGED CULTURE

PART II – EFFECT OF CARBON SOURCE ON PRODUCTION OF POLYGALACTURONASE

K. ZETELAKI-HORVÁTH and K. VAS

(Received January 20, 1971)

The effect of various carbon sources (glucose, maltose, galactose, lactose-fructose, starch, arabinose, xylose, sucrose and pectin) on the growth and pecto. lytic enzyme formation of *Aspergillus awamori* in submerged culture was examined High yields of mycelia and of polygalacturonase (as measured by the decrease in viscosity of pectin solution) were obtained in cultures grown in media containing sucrose, fructose, starch, maltose and glucose.

Apple juice clarifying activity of cultures grown in media containing various carbon sources was very low, the highest concentrations (SPA^{A}_{75} = 139 and 298 l/h/l) being shown by cultures grown in media containing pectin and starch, respectively.

Formation of other hydrolytic extracellular enzymes (cellulase, carboxy-methylcellulase, amylase and maltase) by the mould, as well as the mode of breakdown of polygalacturonic acid by culture filtrates grown in different carbon sources were also examined.

Production of the above accompanying enzymes showed a peak at 24-hour cultivation and dropped to very low levels after 72 hours, while polygalacturonase formation increased considerably throughout the 3 days of cultivation.

The pectolytic enzyme production by plant pathogenic and other micro-organisms has been studied by many authors (PIERSON et al., 1955; SMITH, 1958; TWEEDY & POWELL, 1963).

The metabolism and enzyme formation of different microorganisms is influenced by the composition of the medium. The carbon source requirement of microorganisms varies with the species and the nature of their product. AGNIHOTRI (1962) found glucose, fructose, galactose, xylose, rhamnose and arabinose to be equally good carbon sources for Aspergilli. For glucose oxidase production of *Aspergillus niger*, glucose and sucrose were found to be the best carbon sources (MUNK et al., 1963; FRANKE et al., 1965; ZETELAKI, 1966).

This paper deals with the results of experiments carried out to determine the effect of carbon source on the growth and production of polygalacturonase as well as of the apple juice clarifying agents by *Aspergillus awamori*. The formation of several accompanying extracellular enzymes was also studied.

1. Materials and methods

1.1. Microorganisms

An UV variant (No. 272) of *Aspergillus awamori* was used as the test organism (ZETELAKI-HORVÁTH & DOBRA-SERES, 1972).

1.2. Inoculum

10% of 24-hour vegetative cultures were used for inoculation.

1.3. Composition of basal medium

$(NH_4)_2SO_4$: 6 g, KH_2PO_4 : 2 g, corn-steep liquor: 10 g/1000 ml, pH: 4.0.

As carbon sources 10 g of glucose, maltose, galactose, lactose, fructose starch, arabinose, xylose or Obipektin were used. The media with sucrose as carbon source served as control.

1.4. Cultural conditions

Cultures were incubated for 72-hours on a shaking machine of 330 rpm with a stroke-length of 200 mm.

Oxygen solution rate in the flasks was 17 mmol O_2/l/h as determined by the sulphite oxidation method of COOPER et al. (1944).

The temperature of cultivation was 28 °C.

1.5. Mycelial weight

This was determined after drying the mycelia of the culture to constant weight at 105 °C.

1.6. Polygalacturonase activity

Polygalacturonase activity was measured viscometrically with Ostwald type viscometer, using a 0.25% pectin solution (Obipektin, type: Violettband, Obipektin AG, Bischofszell, Schweiz; the degree of esterification was 33%) as substrate. The decrease in viscosity of the substrate solution was measured and expressed as SPA_{75} (Specific Pectolytic Activity). SPA_{75}^{Obi} indicates the quantity (litre) of substrate solution which is decomposed to reach a loss of its original specific viscosity of 75% by litre culture filtrate, on incubation for 1 hour at 50° (VAS, 1953).

1.7. Evaluation of apple juice clarifying activity

Apple juice clarifying activity was measured by the decrease of the initial specific viscosity of apple juice after incubation with the culture filtrates at 50 °C for 1 hour. The variety of apple used was Staymared with an initial specific viscosity of: 1.550. Concentration of apple juice clarifying agents (SPA_{75}^{A}) was expressed by the quantity of apple juice, the pectin content of which was decomposed to reach 75 per cent loss of its original specific viscosity by 1 litre of filtrate or by 1 kg of mycelia (l/h/l or l/h/kg).

1.8. Evaluation of cellulase (C_1), carboxymethylcellulase (CMC-ase), amylase and maltase activities

The activities of the above enzymes were estimated by the amount of glucose liberated from the substrates of these enzymes (after 1 hour of incubation at 40 °C) and were expressed as micromole glucose per minute (U = μmole/min.).

Substrates used were as follows:

1% suspension of filter paper (Macherey & Nagel Co., No. 640 m) for cellulase; 1% carboxymethylcellulose (Magyar Viscosa, Nyergesújfalu) for carboxymethylcellulase; 1% soluble starch (p.a.) for amylase; and 1% maltose (p.a.) for maltase determination.

Glucose was determined by the method of SOMOGYI (1952).

2. Results

The growth curves of *Aspergillus awamori* cultures grown in media containing different carbon sources are shown in Fig. 1.

After 72 hours of cultivation, high mycelial yields were obtained in the cultures grown in media containing sucrose, fructose, starch, maltose and glucose, while the lowest mycelial yields in those containing arabinose and pectin, resp.

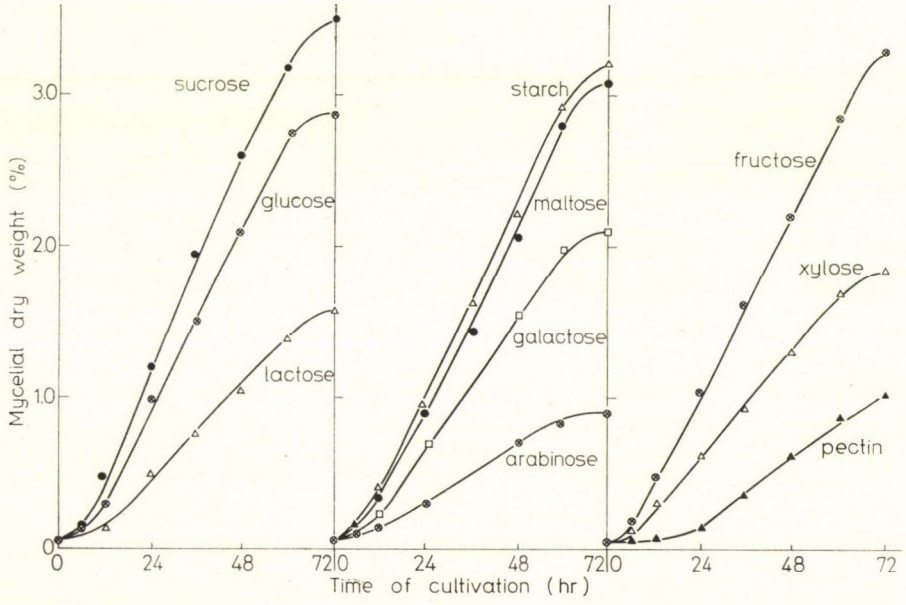

Fig. 1. Growth of shake cultures of *Aspergillus awamori* in media containing different carbon sources

Table 1

Effect of carbon source on growth rate of Aspergillus awamori in submerged culture

Carbon source	x_{max} (g/100 ml)	Standard deviation (s) (g/100 ml)	Rate of growth g/l/h	μ_{max}	t_g
Arabinose	0.91	0.40	0.014	0.160	13.5
Xylose	1.75	0.60	0.028	0.166	12.4
Glucose	2.88	0.22	0.040	0.166	10.2
Fructose	3.29	0.28	0.046	0.110	12.3
Galactose	2.10	0.20	0.033	0.162	10.6
Maltose	3.09	0.18	0.045	0.160	11.1
Sucrose	3.50	0.42	0.055	0.110	11.1
Lactose	1.59	0.12	0.025	0.160	12.9
Starch	3.20	0.26	0.046	0.164	11.8
Pectin	1.02	0.34	0.015	0.150	13.1

$$s = \sqrt{\frac{\Sigma_i(x_i - \bar{x})^2}{n - 1}}$$

x_{max} = maximum mycelial yield
μ_{max} = maximum specific growth rate
t_g = generation time (h)

The maximal specific growth rates and growth rates between the 12th and 24th hours of fermentation, as well as the maximum mycelial yield of the cultures in media containing various carbon sources are shown in Table 1.

Growth rates and specific growth rates were calculated (AIBA et al., 1965) according to equation 1 and 2 resp.

$$\text{growth rate} = \frac{dx}{dt} \qquad 1.$$

$$\text{specific growth rate} = \frac{1}{x}\frac{dx}{dt} \qquad 2.$$

where x = yield of mycelium (g/l),
t = time of cultivation (h).

According to Table 1, maximum mycelial yield and high rates of growth (0.055, 0.046, 0.046, 0.045 and 0.040) were shown by cultures grown in media containing sucrose, fructose, starch, maltose and glucose, respectively, while the lowest rates (0.014 and 0.015 g/l/h) by those grown in media containing arabinose or pectin.

The values of the maximum specific growth rates were 0.110 in cultures grown on fructose and sucrose, while in cultures grown on other carbon sources the maximum specific growth rates were about 0.160.

Generation time values of the cultures grown on various carbon sources were around 10—13 hours.

Comparison of polygalacturonase production of cultures grown in media containing different carbon sources (Fig. 2) showed polygalacturonase yields to be high with sucrose, starch and fructose (SPA_{75}^{Obi} = 4766, 4354 and 4092 l/h/l, resp.).

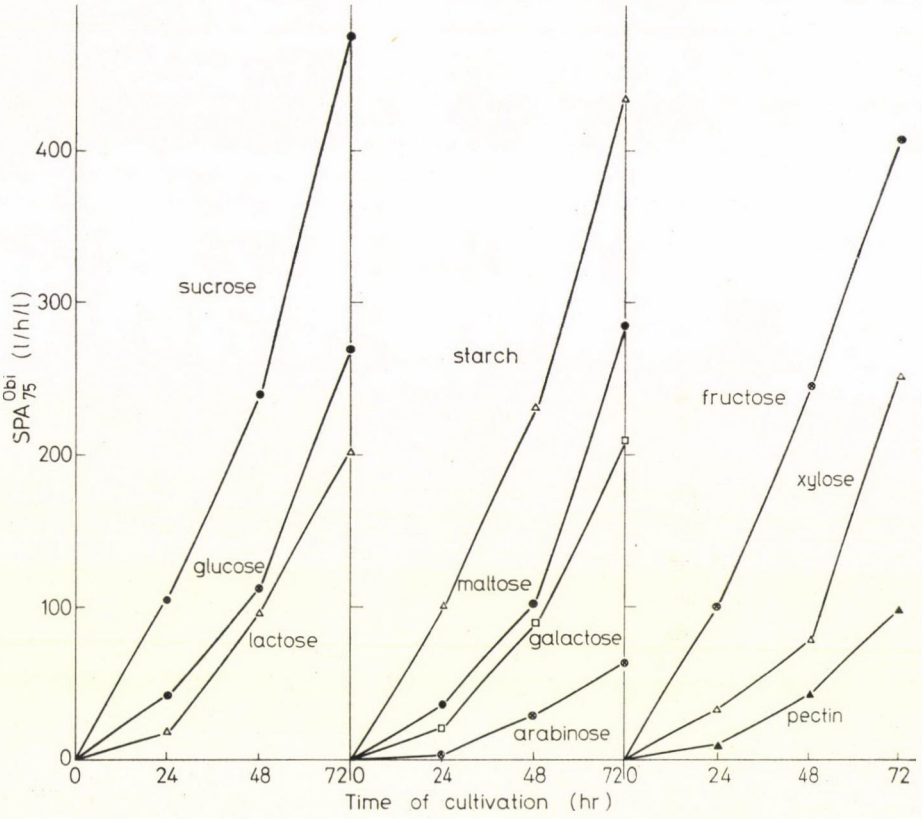

Fig. 2. Polygalacturonase production of shake cultures of *Aspergillus awamori* grown on different carbon sources

Polygalacturonase concentrations of cultures grown in media containing other carbon sources (maltose, glucose, xylose, galactose, lactose or pectin), were lower (SPA_{75}^{Obi}: 2873, 2705, 2553, 2132, 2028 and 1018 l/h/l, resp.).

Cultures grown in media containing various carbon sources were examined also for apple juice clarifying activity. The results are shown in Fig. 3.

As can be seen from Fig. 3 the concentration of apple juice clarifying agents (SPA_{75}^{A}) in the cultures grown in the presence of one of the above mentioned sugars, was in each case less than 20. Using starch or pectin as the carbon

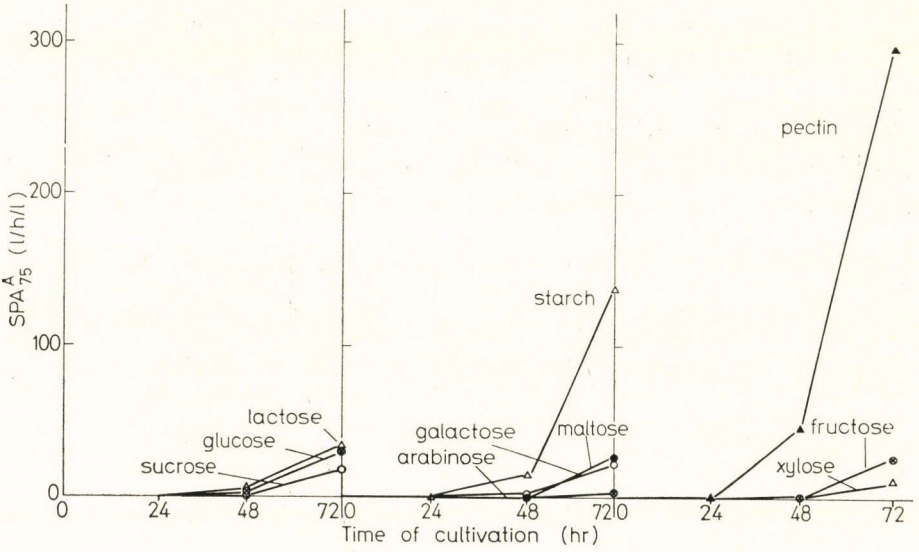

Fig. 3. Concentration of apple juice clarifying enzymes (SPA^{A}_{75}) in the shake cultures of *Aspergillus awamori* grown in media containing various carbon sources

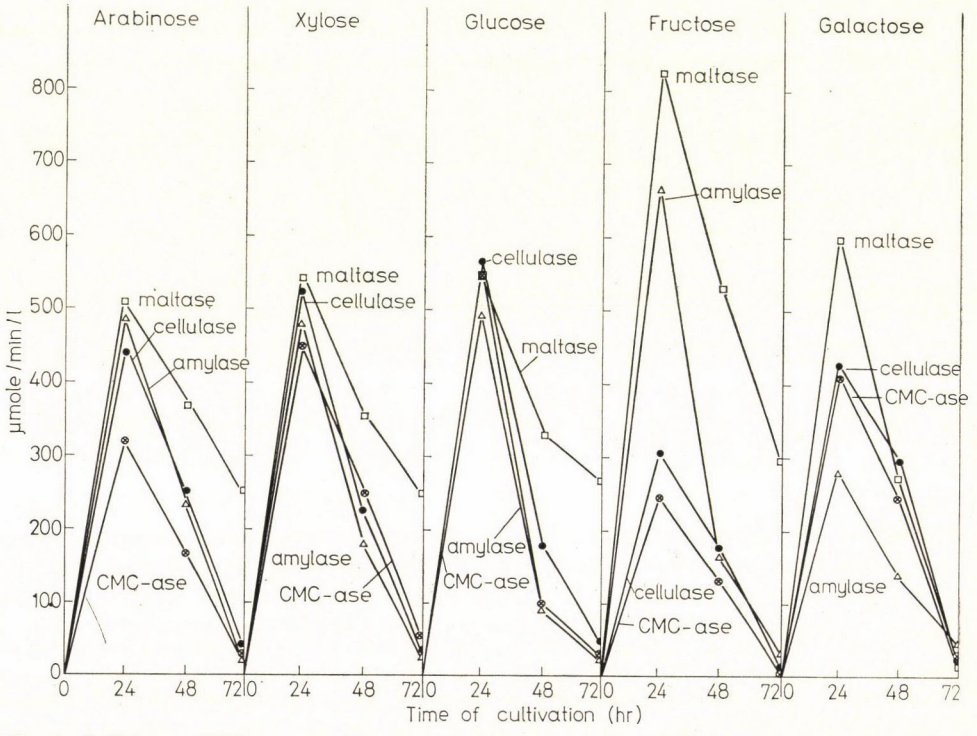

Fig. 4. Cellulase, CMC-ase, amylase and maltase activities of 72-hour shake cultures of *Aspergillus awamori* in media containing arabinose, xylose, glucose, fructose or galactose as the carbon source

source, the activities of the culture filtrates were higher ($SPA_{75}^{A} = 139$ and 297 l/h/l, resp.).

Activities of the accompanying enzymes present in the culture filtrates are shown in Figs 4 and 5.

Obviously, of the accompanying enzymes tested, maltase activity was the highest with almost all carbon sources.

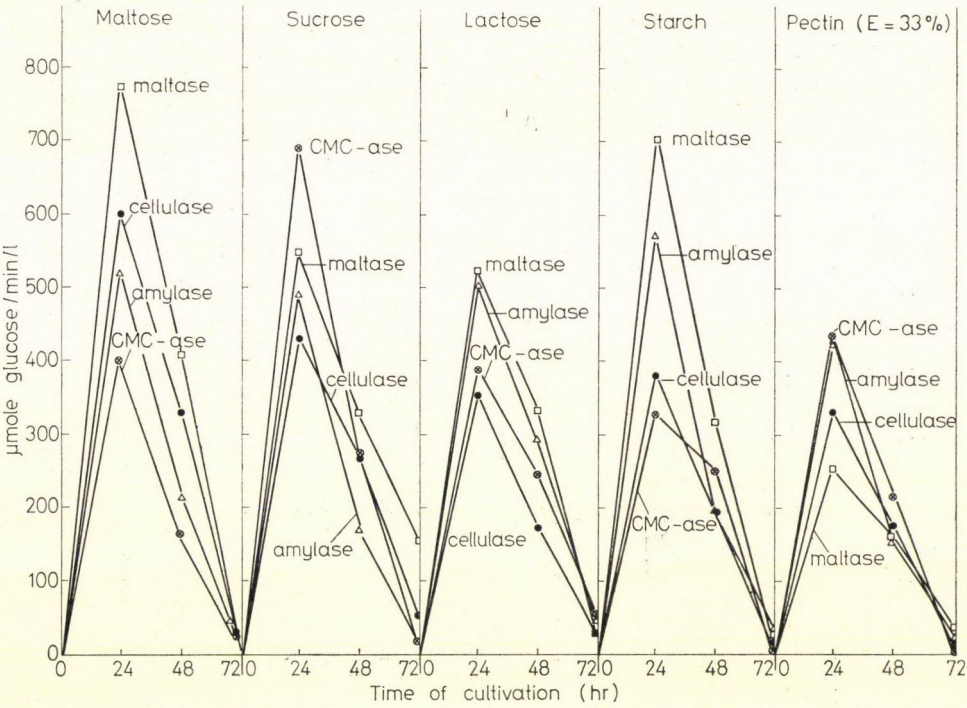

Fig. 5. Cellulase, CMC-ase, amylase and maltase activities of *Aspergillus awamori* in media containing maltose, sucrose, lactose, starch or pectin as the carbon source

The maltase concentrations of 24-hour cultures grown in fructose, maltose, starch, galactose, xylose, glucose, sucrose, lactose, arabinose and pectin, were 820, 770, 700, 600, 540, 545, 550, 520, 510 and 250 µmol glucose/min/l culture filtrate, respectively.

Amylolytic concentrations of the cultures grown for 24 hours in media containing fructose, starch, maltose, lactose, glucose, sucrose, arabinos, xylose, pectin and galactose, resp., were 665, 570, 520, 500, 495, 490, 490, 480, 430 and 280 µmole glucose/min/l culture filtrate, resp.

Cellulase concentrations of cultures grown for 24 hours in media containing maltose, glucose, xylose, arabinose, galactose, sucrose, starch, lactose, pectin and fructose, resp., were 600, 565, 620, 440, 430, 430, 380, 350, 330 and 300 µmole glucose/min/l, resp.

Carboxymethylcellulase concentrations of 24 hours cultures grown in media containing sucrose, glucose, xylose, pectin, galactose, maltose, lactose, starch, arabinose and fructose were 690, 545, 450, 440, 410, 410, 390, 330, 320 and 240 μmole glucose/min/l culture filtrate, respectively.

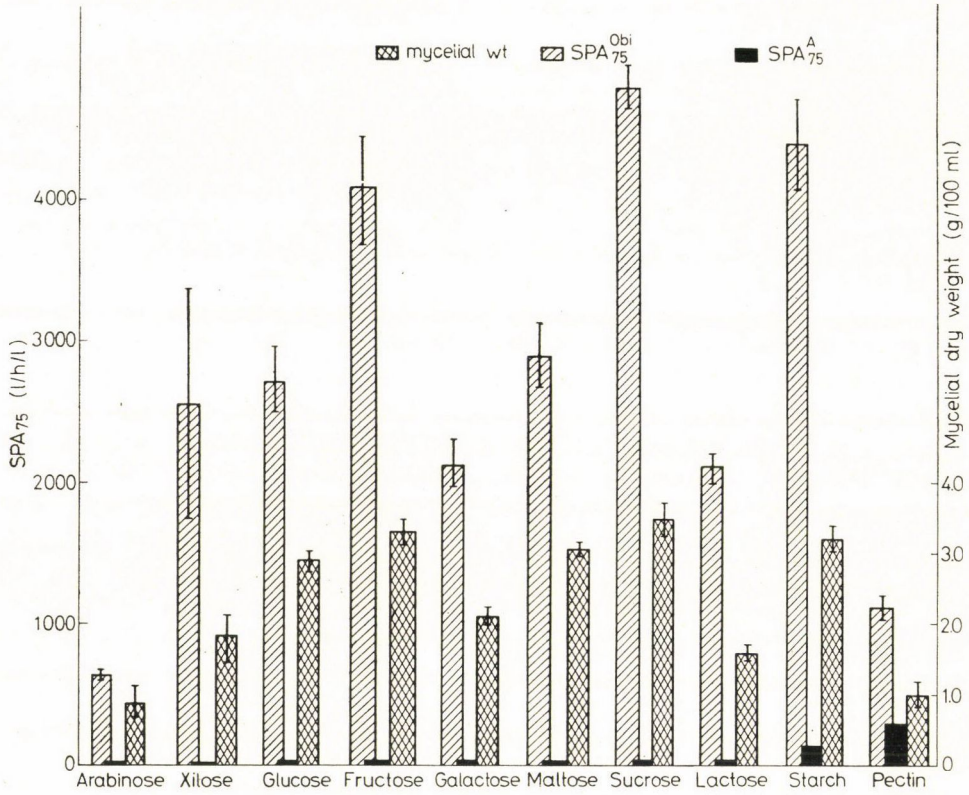

Fig. 6. Yield (means and standard deviations) of mycelia, polygalacturonase (SPA_{75}^{Obi}) and apple juice clarifying enzymes (SPA_{75}^{A}) of 72-hour shake cultures of *Aspergillus awamori* with different carbon sources used in the media

Data obtained for mycelial growth and polygalacturonase production by 72-hour cultures grown in media with various carbon sources are summarized in Fig. 6, while mathematical statistical analysis of the results is presented in Table 2. The diagrams represent the mean values and standard deviations for eight shake cultures.

Fig. 6 shows that sucrose, fructose, starch, maltose, and glucose were good carbon sources for the growth and polygalacturonase production of the mould, while the apple juice clarifying activities of the culture were highest with pectin and starch as carbon sources.

The mode of action of the polygalacturonase-containing culture filtrates incubated with various carbon sources was investigated chromatographically using polygalacturonic acid (with a degree of esterification of 3.5%) as the substrate (Fig. 7).

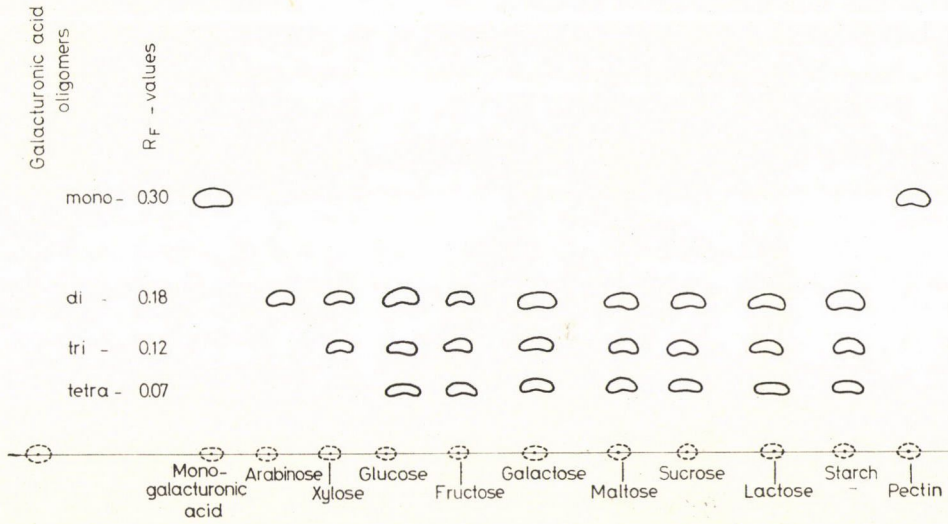

Fig. 7. Chromatographic patterns of the degradation of pectic acid (degree of esterification: 3.5%) by filtrates of *Aspergillus awamori* cultures grown in media containing different carbon sources

Table 2

Mathematical-statistical analysis of the polygalacturonase (SPA_{75}^{Obi}) production by Aspergillus awamori cultures grown on different carbon sources in submerged culture for 72 hours

C-source	Mean SPA_{75}^{Obi} (l/h/l)	Standard deviation (l/h/l)	Comparison with results on sucrose		Degree of freedom (DF)
			t	P%	
Arabinose	655	39	37.6	99.9	12
Xylose	2553	1647	2.97	99	14
Glucose	2705	476	10.0	99.9	15
Fructose	4092	859	1.96	95	15
Galactose	2132	219	22.0	99.9	15
Maltose	2873	476	9.2	99.9	15
Sucrose	4766	278	—	—	
Lactose	2028	202	23.7	99.9	15
Starch	4354	641	1.56	95	14
Pectin	1019	81	25.74	99.9	11

Fig. 7 shows the enzymatic degradation of polygalacturonic acid to be similar for the filtrates of cultures grown in different carbon sources.

Filtrates of cultures grown in media containing glucose, maltose, galactose, lactose, fructose and sucrose degraded polygalacturonic acid into di-, tri- and tetragalacturonic acids. Only four types of culture filtrates differed from the rest in polygalacturonic acid degrading effect.

The filtrates of cultures grown in starch produced di-, tri- and tetragalacturonic acids, while arabinose media gave only digalacturonic acid spots on the chromatogram, and xylose media resulted in tri- and tetragalacturonic acids. The filtrates of the cultures grown in pectin-containing media broke down polygalacturonic acid into monogalacturonic acid.

3. Discussion

The effect of the carbon source on the pectolytic activities of various microorganisms has been investigated by many authors. CHANDRA and TANDON (1963) applying glucose, fructose, galacturonic acid or citrus pectin as the carbon source for *Aspergillus terreus*, found pectin to be the best for pectinmethylesterase, polygalacturonase and pectindepolymerase production. NYESTE and HOLLÓ (1963) used glucose, starch and pectin, while VASU (1967) applied sucrose and pectin for the pectolytic enzyme formation of *Aspergillus niger*, BALKAY and VAS (1968) found glucose satisfactory as a carbon source for the polygalacturonase production of *Aspergillus foetidus*.

The present study of the growth and polygalacturonase formation of *Aspergillus awamori* over the entire growth cycle showed some correlation between the yield of mycelia and polygalacturonase activity. When polygalacturonase production in 72-hour cultures is plotted against yield of mycelia grown on various carbohydrates, Fig. 8 is obtained.

As can be seen in Fig. 8, a fairly close positive correlation existed between growth and concentration of polygalacturonase acting on commercial pectin preparations (Obipektin). Production of apple juice clarifying agents, on the other hand, could not be correlated with mycelial growth. Pectin, giving a low mycelial yield, showed the highest enzyme concentration while starch, supporting heavy growth of mycelia, was the second best carbon source to produce apple juice clarifying enzymes. The concentrations of polygalacturonase and of apple juice clarifying agents are, thus, apparently unrelated.

The cultures grown in sucrose, starch, fructose, maltose and glucose had the highest yield of mycelia and showed significantly higher polygalacturonase activity ($P > 99.9\%$) than those grown in galactose, lactose, arabinose, or pectin (Tables 1 and 2).

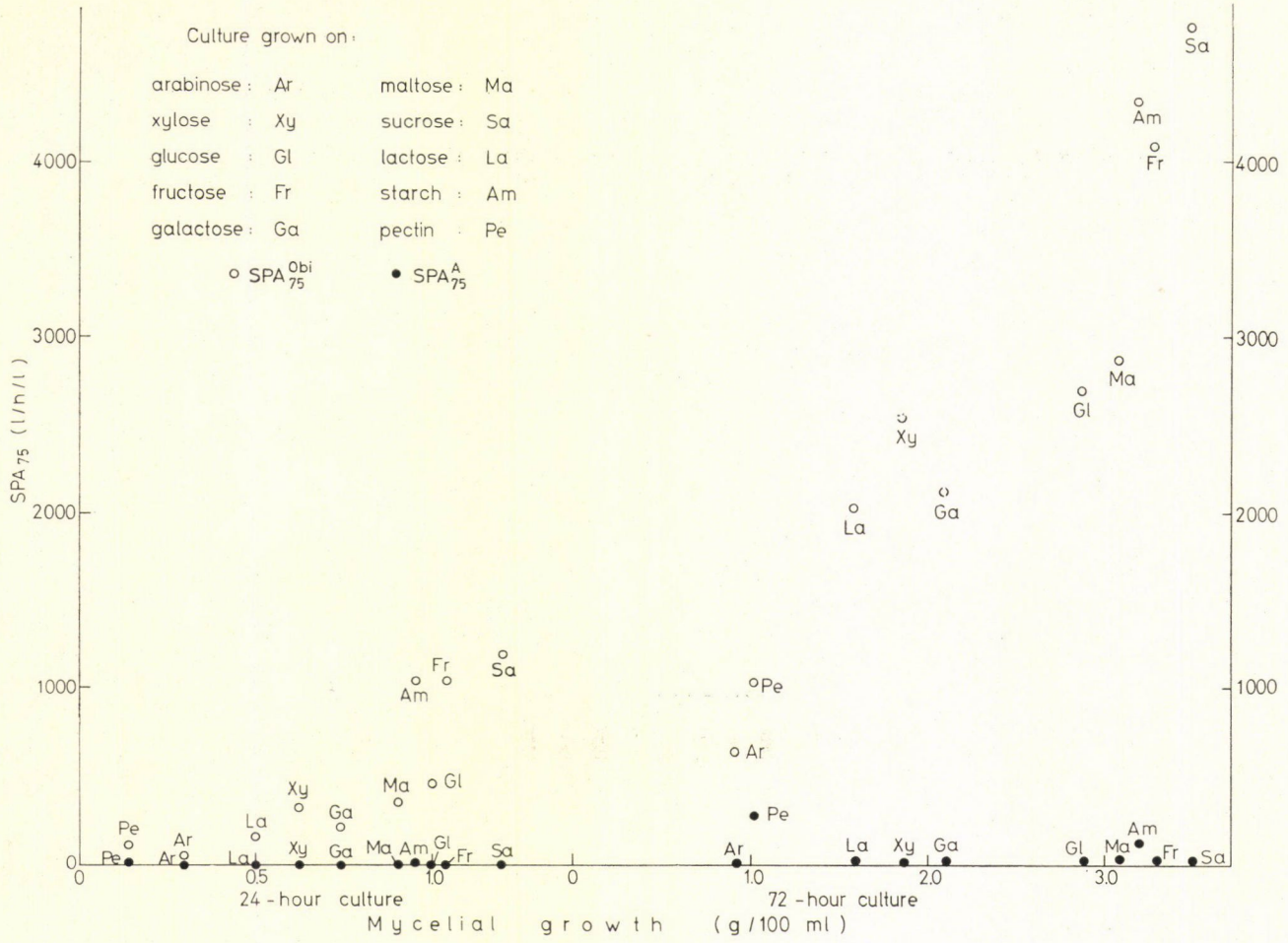

Fig. 8. Polygalacturonase formation as a function of mycelial yield in cultures of *Aspergillus awamori* grown on various carbon sources

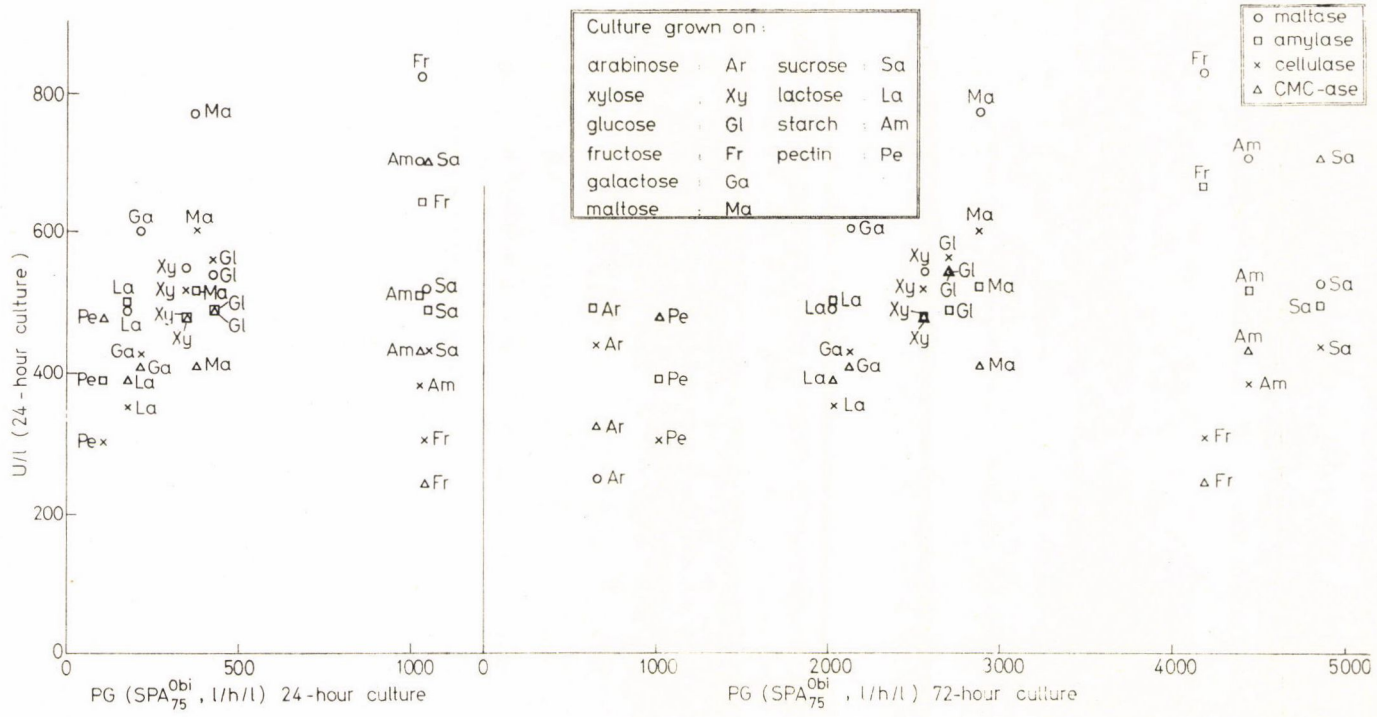

Fig. 9. Correlation between production of polygalacturonase and formation of maltase, amylase, cellulase and carboxymethylcellulase, resp. in cultures of *Aspergillus awamori* grown in media containing various carbon sources

Table 3

The growth and enzyme formation of Aspergillus awamori when grown in media containing different carbon sources

	Age of culture (hrs)	Enzyme contentrations in cultures grown in solutions containing as the carbon source									
		arabinose	xylose	glucose	fructose	galactose	maltose	sucrose	lactose	starch	pectin
Maltase	24	510	540	545	820	600	770	550	520	700	255
(U/l)	72	250	250	270	295	19	10	155	18	20	36
Amylase	24	490	480	495	665	280	520	490	500	570	430
(U/l)	72	20	30	22	30	42	42	10	40	30	20
Cellulase	24	440	520	565	300	430	600	430	350	380	330
(U/l)	72	40	38	50	15	30	30	50	25	10	15
CMC-ase	24	320	450	545	240	410	410	690	390	330	440
(U/l)	72	30	58	30	10	30	20	15	50	5	5
PG (Obi)	24	50	350	430	1040	220	380	1050	180	1030	110
(l/h/l)	72	655	2553	2705	4092	2132	2873	4766	2028	4354	1019
PG (apple)	24	—	—	—	—	—	—	—	—	2	4
(l/h/l)	72	4	12	31	27	23	28	19	35	138	290
Mycelial growth	24	0.30	0.62	1.00	1.04	0.74	0.90	1.20	0.50	0.95	0.14
(g/100)	72	0.90	1.84	2.88	3.28	2.10	3.08	3.50	1.59	3.20	1.02
Growth rate (g/l/h)	12—24	0.014	0.028	0.040	0.046	0.033	0.045	0.055	0.025	0.046	0.015
Breakdown products of pectin*	72	2	3, 4	2, 3, 4	2, 3, 4	2, 3, 4	2, 3, 4	2, 3, 4	2, 3, 4	2, 3, 4	1

* 1: mono- galacturonic acid; 2: di-galacturonic acid; 3: tri-galacturonic acid; 4: tetra-galacturonic acid; 5: penta-galacturonic acid.

Our results are in agreement with those of YAMASAKI and co-workers (1966) who obtained a very high endo-polygalacturonase yield on sucrose media without adding pectin.

Production of other hydrolytic extracellular enzymes did not notably vary with the different carbon sources. Our results concerning the production of amylase are inconsistent with those of FENIKSOVA (1957) who found no amylase production in cultures of *Aspergillus oryzae* grown in any carbon source but starch, dextrose or maltose. *Aspergillus awamori* showed amylase activity in every carbon source used, consistently with the results of COLEMAN (1967).

Cellulase activity of the culture filtrates was highest when the growth media contained maltose, glucose or xylose as the carbon source, while carboxymethylcellulase activities were optimal with sucrose, glucose, or xylose used as carbon sources. WHITNEY and co-wokers (1969) used cellulose or carboxymethylcellulose powder in the media of *Verticillium albo-atrum* to enhance carboxymethylcellulase production.

We found maltase in all *Aspergillus awamori* cultures grown in media containing the above carbon sources. High maltase concentrations were obtained in fructose, maltose and starch media, while the lowest was found in a medium containing pectin as the carbon source.

Data of growth and enzyme formation of the cultures grown in media containing various carbon sources are summarized in Table 3.

Some correlation between polygalacturonase production and the formation of some other hydrolytic extracellular enzymes could be detected (Fig. 9).

It has to be made clear, however, that even this weak correlation can only be shown in young cultures. After 24 hours of cultivation, maltase, amylase, cellulase and CMC-ase concentrations drop very steeply and reach almost insignificant levels after 72 hours (Figs 5 and 6). This phenomenon can be made use of when the production of polygalacturonase relatively free of accompanying enzymes is aimed at.

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TEST METHODS AND EVALUATION PROCEDURES FOR THE DETERMINATION OF THE SENSORY PROPERTIES OF FOODSTUFFS

T. SÁRAY, GY. URBÁNYI and E. DOBRAY-HORVÁTH

(Received January 4, 1971)

The experiments included samples of radiation treated dried French beans, sour cherry juice and mushroom paste, tested by a panel of 12 tasters using scoring and difference test methods of sensory analysis. To increase the objectivity of sensory judgement, an attempt was made to evaluate the data characteristic of the more important parameters of the products by methods of mathematical statistics.

In the scoring type sensory tests on dried French beans, irradiated with 0.15, 0.60 and 2.40 Mrad doses and cooked for 5, 10 and 15 minutes, respectively, when prepared for the test, it was found that the sample irradiated with 2.40 Mrad does not differ from the other samples as far as odour, taste and texture are concerned, but has a significantly different colour when evaluated by Kramer's well known method.

In the comparison of the colour of the samples, using again Kramer's evaluation method, a significant difference was found between the samples irradiated with 0.15 Mrad and the control when 10- and 15-minute cooking periods were used.

In the case of French beans the differences in the standard deviations of the scores for the majority of tested parameters could not be statistically confirmed by means of the variance ratio test. The same is true for the taste scores of sour cherry juice samples.

Comparison of the mean values of food samples according to a given scoring scale is only possible if the samples are judged independently from one another. This condition is but rarely fulfilled and therefore the colour, odour and taste scores of dried French bean samples were in this experiment evaluated also by t test used for the comparison of the averages of paired values.

No significant difference was found by analysis of variance in the colour of dried French bean samples, or in the taste and odour of sour cherry juices when the time of judgement was taken into consideration.

Deviations of scores between samples and between panelists are significant to different degrees. Unequivocal interaction was found only in the colour judgement of French beans, in other cases the interaction between the various factors could not be proven unambiguously.

Deviations in the taste of sour cherry juice and mushroom paste samples were investigated by the triangular and duo-trio difference tests. In the case of sour cherry juice the deviations from the control are — similarly to scoring tests — very high. Similar results were obtained in duo-trio tests, though the significance was lower.

It was found in the difference tests performed with mushroom pastes of different mushroom contents that while in the triangle tests a significant difference was found by the panelists for each trial, the difference could not be confirmed by duo-trio tests. The reason for this might be found in the impossibility of repeated tasting.

In the last 100 years organoleptic analysis developed into an almost independent discipline and became an indispensable tool in the qualification of food.

Related research developed in several directions, but in every case it was aimed at developing sensory evaluation into a reliable scientific method furnishing reproducible data.

Several authors have voiced the opinion (SPANYÁR, 1958; TELEGDY KOVÁTS, 1955; TILGNER, 1958; AMERINE et al. 1965) that in the complex investigation of foodstuffs, beside the physical, chemical and instrumental determinations, tasting and smelling by man cannot be replaced.

Sensory methods of judgement have undergone considerable development in the past 20 years and have more or less taken shape (TELEGDY KOVÁTS, 1967; VAJDA, 1969; KIERMEIER & HAEVECKER, 1969).

Appropriate mathematical statistical methods helped to improve the objectivity and reproducibility of sensory tests. AMERINE et al. (1965), KÖRMENDY (1964) and VAJDA (1969) have presented surveys of the pertaining methods. In the evaluation of sensory tests ranking, difference tests, analysis of variance, hypothesis testing, correlation and regression calculations, etc. are currently used.

In the evaluation of scoring methods rank correlation might be applied when the original data are replaced by rank numbers and the data obtained are then compared. In this way the level of significance can be rapidly calculated by means of KRAMER's method (1956, 1960). KOCHAN (1965) subjected difference tests to a critical analysis. He described the triangular test which has the great advantage of being easy to evaluate. In arranging the triangle test the probability of a chance occurrence of the observed difference can be examined with the desired reliability with the help of the χ^2 test. The paired comparison of rank correlations promises to be a safe method (KOZMA, 1961). This determines the coefficient of concordance and performs evaluation on the basis of scores obtained by comparison with the help of the above coefficient.

TELEGDY KOVÁTS (1967) carries out comparison between paired samples. For significance tests he uses t, χ^2, and F test calculations. He suggests for preference tests the duo-trio test. For the evaluation of this test the principle of critical ratio is suggested by PERYAM and SCHWARTZ (1950). According to these authors in preference tests information of the desired accuracy necessitates a certain number of responses, for which, however, the permissible limit of error and the level of significance must be known.

Comparison of food samples according to hedonic or point scales on the basis of average values by means of Student's general t test will be possible only if the samples are judged independently from one another (STREULI, 1967). In the case of sensory tests this condition is but seldom fulfiiled, since in most cases the various samples are judged by the same panelists. In this case the t test has to be performed by comparing the averages of paired data.

KIERMEIER and HAEVECKER (1969) described sequential analysis in full

detail, since it is of particular importance in the choice of suitable panelists and for shortening test procedures.

ÖRSI (1968) dealt with methods of sensory qualification of commercial goods. He used analysis of variance to decide whether the calculated discrimination function was suitable for the distinction of two quality classes. ZUKÁL (1955) performed identification and deviation tests and judged dilution series. He listed the distribution between the numbers of right and false identifications in a so-called contingence table. Further he carried out preference tests, too. The ranking rule observed in simple tests, according to which the panelist is capable of ranking extreme values with a greater certainty than the average values, is valid for complete tests, too. Results obtained in several repetitions occurred in general with a frequency corresponding to normal distribution. ZUKÁL and co-workers (1963, 1964) studied also the distribution of sampling and qualification. They believe that in the evaluation of sensory judgements the calculation of distribution values might be successful. When drawing conclusions from the sample on the batch the concepts of quality and the correlations between sample and batch have to be analyzed. They calculate the qualifying limit, or the value compared to which the average of the sample must fall into the favourable direction.

Information gained by mathematical statistical methods must be critically accepted and judiciously used, though beyond all doubt this is the only way to obtain realistic information.

There is always a great need for the determination of the organoleptic properties characterizing and numerically expressing the sensory value of food. The up-to-date trend in testing requires scientifically founded judging methods, from the qualification of tasters through the proper application of the various testing methods to the extensive evaluation of the data. In the interest of proper utilization of the data furnished by this subjective method it is necessary to subject the "opinion" of the individual tasters to mathematical evaluation.

1. Materials and methods

Of the many methods of sensory analysis for research purposes we have chosen scoring ranking, being one of the quality estimation methods frequently used because of its simplicity and versatility. The method lends itself to statistical evaluation. The colour, odour, taste and texture of dried French bean samples having been subjected to different doses of radiation and to boiling and put at our disposal by the Central Food Research Institute, were judged by us using a maximum 10 point scale. (The first digit in the number of the sample stands for: 1 = control, 2 = 0.15 Mrad, 3 = 0.60 Mrad, 4 = 2.40 Mrad radiation dose, the second digit stands for: 1 = 5 minutes, 2 = 10 minutes, 3 = 15 minutes cooking.)

The odour, colour and taste of the four sour cherry juice samples prepared at our Department were evaluated in a similar manner. (1 = control, directly prepared, clarified and filtered juice, 2 = aroma obtained by cold stripping + concentrate stripped of aroma, rediluted, 3 = aroma obtained by hot stripping + concentrate stripped of aroma, rediluted, 4 = sour cherry juice rediluted from concentrate.)

Both products were judged by the same panel at the same time.

The data were evaluated in different ways. Thus the calculated standard deviation values for the various samples were used to carry out the F tests comparing the deviations between the samples. On the bases of these Student's general t test was performed. The obtained t values were then subjected to significance test.

If the standard deviation of the samples differed significantly (the F test was significant) the value of t was calculated from the modified equation.

However, a stochastical independence between the ranking series is one of the important conditions for the applicability of the general t test. This condition is usually not met even under optimum conditions of testing since in most cases every panelist scores all the samples so that the bulk of given scores is essentially related. For this reason to evaluate the differences obtained from the scores by the Student test alone is not correct. According to KÖRMENDY (1964), AMERINE and co-workers (1965) and STREULI (1967) in this case the difference test is the most appropriate mathematical procedure, that is the application of the t test based on the comparison of the averages of paired data.

$$t = \frac{\Sigma D}{\sqrt{\frac{N \cdot \Sigma D^2 - (\Sigma D)^2}{N - 1}}}$$

(Manual of Sensory Testing Methods, 1968)

In our experiment we have used this recommended procedure in order to ascertain whether the significant deviations are in fact caused by the applied treatments or by some other external influence.

Next we have used Kramer's customary evaluation method when the sums of rank scores obtained by ranking were compared and evaluated by means of Kramer's Table.

In order to clarify the relationships and to obtain further information the judgements by scoring were evaluated by analysis of variance, too, on the basis of the results of preliminarily examined relatively homogeneous deviations and relatively normal distribution.

The sensory tests carried out by means of difference tests constituted the other part of our investigation. Of the so-called three sample procedures we used the triangular and duo-trio tests. Analysis of significance was per-

formed by means of the generally used Tables. In the difference tests various sour cherry juice samples and samples of mushroom paste prepared at the Department were judged; discrimination was based on the taste of the samples. The composition of the panel was the same as the one which performed judgement by scoring.

In most cases the panel consisted of 12 tasters chosen from the staff of the Department. First every taster had to pass a test with respect to the threshold of sensing and discrimination using model solutions and original food. Provision was made for adequate storing and preparation of the samples. A complete separation of the panelists was unfortunately possible only in the case of the difference tests. Testing took place under exclusion of all external irritation, at 20° to 21 °C ambient temperature, in the same hour of the day, before the main meal.

2. Results

2.1. Organoleptic tests using the scoring method

By using Kramer's method the sensory evaluation of French bean samples which have received 0.15, 0.60 and 2.40 Mrad doses, respectively, and in the course of their preparation for testing have been cooked for 5, 10 or 15 minutes, has shown no significant difference in the odour, taste and texture of the product, but with respect to colour the sample treated with 2.40 Mrad differed significantly from the others. From the obtained data an answer was sought to the question how far the samples cooked for different periods and compared in pairs differ with respect to colour, taste and texture. Here again Kramer's method of evaluation was used (Table 1).

With respect to colour there is, in the majority of cases, a statistically confirmed difference between the samples. The difference in colour is particularly marked between the control and the sample treated with 2.40 Mrad, when cooked for 10 or 15 minutes and the samples treated with 0.15 Mrad and 2.40 Mrad, respectively. Comparison of paired samples showed no significant difference between the taste and texture of the control and radiation treated samples.

In the interest of a more concise mathematical statistical analysis the deviation between the colour, odour and texture of the dried French bean samples as a function of the period of cooking was evaluated on the basis of the scores. The standard deviation in the scores given for colour ranged between 0.66 and 1.62, that for taste and texture between 1.04 and 2.21, and 1.11 and 2.39, respectively. By means of the variance ratio test with the exception of a few tests, it was not possible to confirm a significant difference in the standard deviations.

Table 1

Evaluation of sensory tests of dried French bean samples by means of Kramer's method

Date of evaluation: October, 1969
Number of panelists: 12

Tested parameters	Tested samples																	
	11 : 21	11 : 31	11 : 41	21 : 31	21 : 41	31 : 41	12 : 22	12 : 32	12 : 42	22 : 32	22 : 42	32 : 42	13 : 23	13 : 33	13 : 43	23 : 33	23 : 43	33 : 43
	Rank sums and levels of significance																	
Colour	16.0	16.5	14.5	17.0	14.5	14.0	19.5	17.0	12.0	15.5	13.0	14.0	17.0	14.5	12.0	15.5	12.5	14.5
	20.0	19.5	21.5	19.0	21.5	22.0	16.5	19.0	24.0	20.5	23.0	22.0	19.0	21.5	24.0	20.5	23.5	21.5
	—	—	*	—	*	*	—	—	**	—	**	*	—	*	**	—	**	*
Taste	17.5	16.0	17.5	15.5	17.5	18.5	17.0	14.5	17.5	16.5	18.5	21.0	16.0	16.0	17.0	17.0	19.5	19.5
	18.5	20.0	18.5	20.5	18.5	17.5	19.0	21.5	18.5	19.5	17.5	15.0	20.0	20.0	19.0	19.0	16.5	16.5
	—	—	—	—	—	—	—	*	—	—	—	—	—	—	—	—	—	—
Texture	16.0	15.0	18.5	17.0	19.5	20.0	17.0	15.5	19.0	16.0	19.0	19.5	17.5	16.5	17.0	18.0	18.5	18.5
	20.0	21.0	17.5	19.0	16.5	16.0	19.0	20.5	17.0	20.0	17.0	16.5	18.5	19.5	19.0	18.0	17.5	17.5
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

The lowest and highest, significantly non-different rank sums according to KRAMER (1960): 15 and 21 (P = 95%) and 14 to 22 (P = 99%)

— = not significant
* = significant at 95% level
** = highly significant at 99% level

The first digit in the number of samples: 1 = control, 2 = irradiated with 0.15 Mrad, 3 = irradiated with 0.60 Mrad. 4 = irradiated with 2.40 Mrad.

The second digit in the number of samples: 1 = 5 minutes cooking period, 2 = 10 minutes cooking period, 3 = 15 min. cooking period.

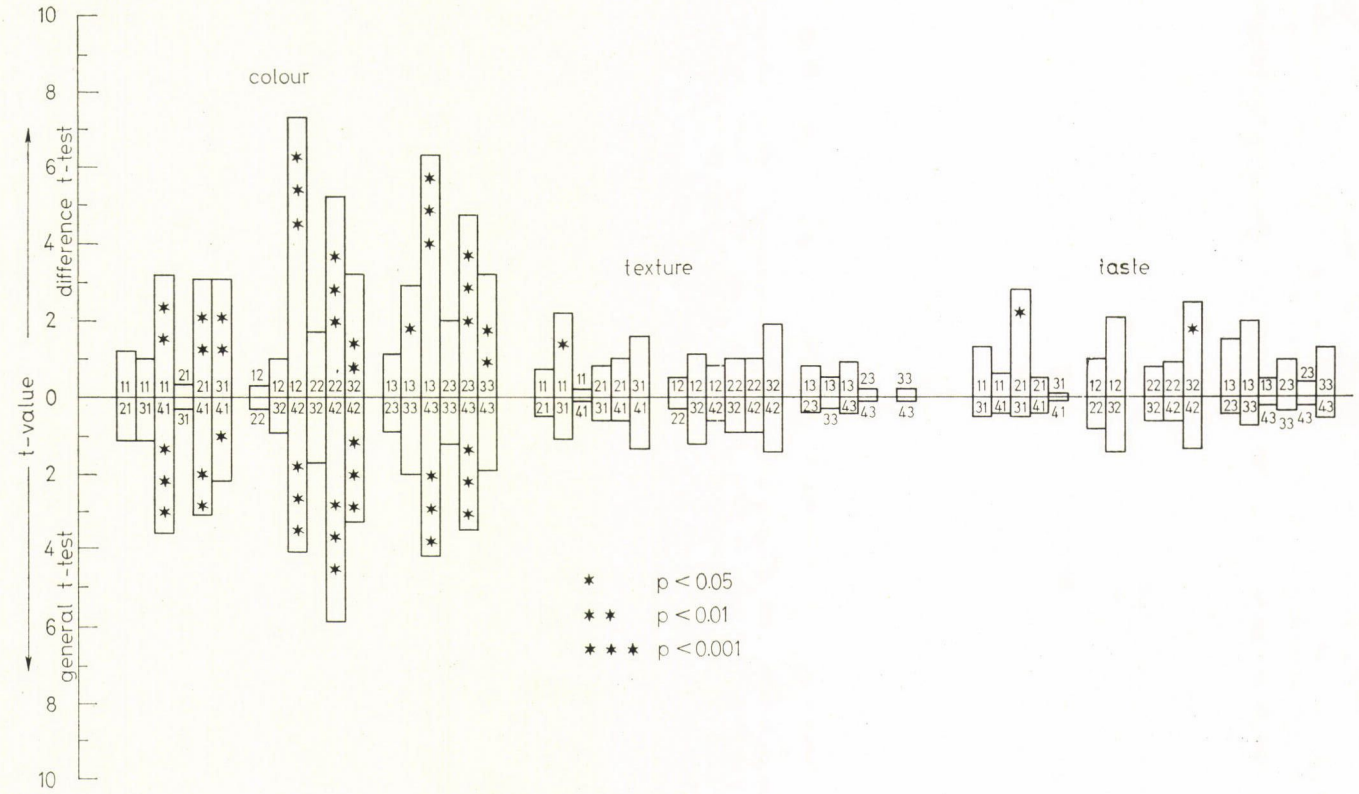

Figure 1. Evaluation by *t*-test of the scores given to the colour, texture and taste of dried French bean samples subjected to various radiation doses and cooking periods (First digit in the number of the sample: 1 = control, 2 = irradiated with 0.15 Mrad, 3 = irradiated with 0.60 Mrad, 4 = irradiated with 2.40 Mrad; second digit: 1 = cooked for 5 minutes, 2 = cooked for 10 minutes, 3 = cooked for 15 minutes.)

The results obtained by applying the generally known Student t test and the difference t test to the comparison of the scores given for the various parameters of paired dried French bean samples are plotted in Fig. 1.

There is a highly or very highly significant difference according to both methods of calculation between the colours of the control and the sample treated with 2.40 Mrad, and the colours of the samples treated with 0.15

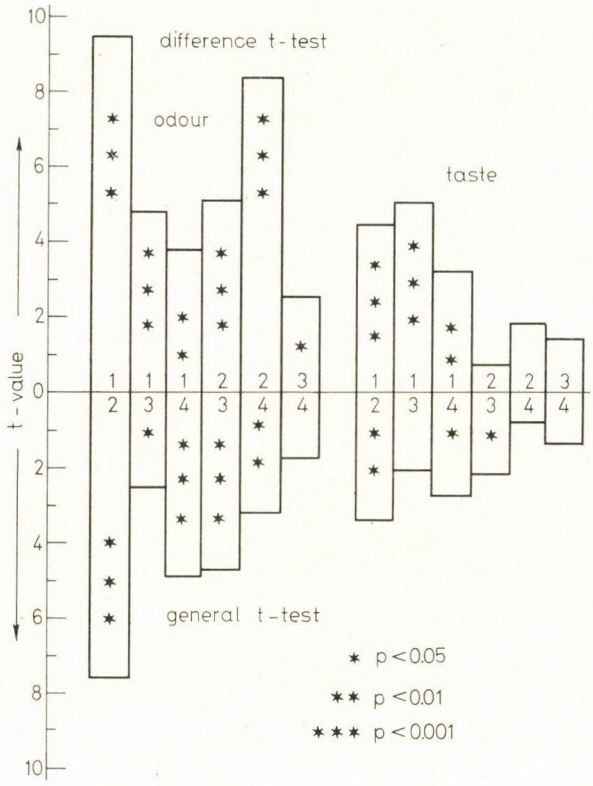

Figure 2. Evaluation by t-test of the scores given to the taste and odour of various sour cherry juice samples (1 = control directly manufactured, clarified, filtered juice, 2 = cold processed aroma + concentrate stripped of aroma, rediluted, 3 = hot processed aroma + concentrate stripped of aroma, rediluted, 4 = sour cherry juice obtained from concentrate by dilution.)

and 2.40 Mrad, respectively, when cooked for 5, 10 or 15 minutes. The t values of the difference test, if the direction of deviation from the average is also accounted for, indicate a significant difference in two cases (between the control and the sample treated with 0.60 Mrad, and those treated with 0.60 and 2.40 Mrad after cooking for 15 minutes) when no such difference is shown by the general t test. The level of significance in the individual points is identical or higher, in favour of the difference t test. But for a few exceptions,

the slight differences in texture and taste of the samples cannot be confirmed with this method either.

The results of the t test analysis (in general the F test shows no significant deviation) of a similar sensory evaluation of the odour and taste of various sour cherry juice samples are given in Fig. 2.

Practically, according to both methods of calculation there is a significant difference in the odour of the samples, the difference being apparent only in the level of significance.

Testing of the taste revealed no significant difference between samples No. 2 (sour cherry juice treated with aroma concentrate prepared by the cold method) and No. 4 (sour cherry juice gained by dilution of the concentrate) or between samples No. 3 (sour cherry juice treated with aroma concentrate prepared by the hot method) and No. 4.

In the course of the sensory food tests we have also used analysis of variance to evaluate the results of scoring and in order to promote the clarification of relationships and to obtain further information. Analysis of variance was based on preliminary experiments which revealed relatively homogeneous deviations and relatively normal distribution, and were destined to decide how far the time of judging, the samples and the members of the panel might be responsible for the differences, if the period of cooking was not considered.

Table 2 shows the result obtained by three-factor analysis of variance of the scores given to the colour of dried French bean samples.

Table 2

Analysis of variance of the sensory testing of some dried French bean samples with respect to colour

Date of testing: October 1969

Source of variance	Sum of squares	Degree of freedom	Variance	F-value	Level of significance
time (A)	1.68	1	1.68	2.02	—
sample (B)	101.83	11	9.26	11.16	***
panelists (C)	146.75	11	13.34	16.07	***
AB	39.99	11	3.64	4.39	***
AC	56.90	11	5.17	6.23	***
BC	172.42	121	1.43	1.72	**
error	100.43	121	0.83		
total	620.00	287			

for the F test:
— = not significant at $F_{5\%}$
** = highly significant at $F_{1\%}$
*** = very highly significant at $F_{0.1\%}$

The efficiency of judging was not influenced by the date of the tests, since the differences were not significant, but the difference between the tested samples and the scores given by the panel are very highly significant. Interactions between the three factors are also highly significantly confirmed.

The results of the analysis of variance of the scores given for the texture of dried French bean samples are given in Table 3.

Table 3

Analysis of variance of the sensory testing of some dried French bean samples with respect to texture

Date of evaluation: October 1969

Sources of variance	Sum of squares	Degrees of freedom	Variance (s^2)	F-value	Significance
time (A)	0.17	1	0.17	9.12	***
sample (B)	33.26	11	3.02	1.94	*
panelists (C)	550.51	11	50.05	32.29	***
AB	16.12	11	1.47	1.05	—
AC	83.21	11	7.56	4.87	***
BC	173.20	121	1.43	1.08	—
error	187.00	121	1.55		
total	1043.47	287			

For the F test:
— = not significant at $F_{5\%}$
* = significant at $F_{5\%}$
** = highly significant at $F_{1\%}$
*** = very highly significant at $F_{0.1\%}$

At the level of the above factors the situation is somewhat different in the last case. The two different dates of judging caused a very highly significant difference, while the difference in the texture of samples has been confirmed only at 5% probability level. We believe that the analysis of interaction supports the very high significance caused by the date of testing and by the significant differences between panelists. No other interaction could be statistically proved.

Of the many conditions improving the objectivity of sensory tests it is often quite difficult to ensure an adequate number of panelists.

We have, therefore, extended our investigations to this problem, too. With the help of Kramer's evaluation method and with respect to the colour, odour and taste of sour cherry juice samples we investigated the efficiency of the work of a panel of fewer (e.g. six) but highly qualified tasters with proper sense as compared to that of a panel of the desirable size (12 to 20 people) (Table 4).

Table 4

Correlation between the number of panelists and the ranking of some sour cherry juice samples on the basis of Kramer's ranking method

	Symbols of samples							
	1		2		3		4	
	A	B	A	B	A	B	A	B
	Colour							
1st day 12 panelists	20.5	*	33.5	—	31.0	—	35.0	—
2nd day 12 panelists	21.5	—	28.5	—	35.0	—	35.0	—
1—2 days 2×6 panelists	20.0	*	31.5	—	35.0	—	33.5	—
1—2 days 2×6 panelists	22.0	—	30.5	—	31.0	—	36.5	—
	Odour							
1st day 12 panelists	16.0	**	47.5	**	21.5	—	35.0	—
2nd day 12 panelists	14.5	**	47.0	**	25.5	—	33.0	—
1—2 days 2×6 panelists	16.0	**	47.0	**	22.5	—	34.5	—
1—2 days 2×6 panelists	14.5	**	46.5	**	24.5	—	33.5	—
	Taste							
1st day 12 panelists	16.0	**	43.0	**	25.0	—	36.0	—
2nd day 12 panelists	18.5	**	38.0	—	28.5	—	35.0	—
1—2 days 2×6 panelists	14.5	**	40.0	*	29.5	—	36.0	—
1—2 days 2×6 panelists	20.0	*	41.0	*	24.0	—	35.0	—

A = rank sums
B = level of significance
The lowest and highest, significantly not different rank sums according to KRAMER (1960): 21 and 39 (P = 95%) and 19 to 41 (P = 99%)
— = not significant
* = P = significant at 95% level
** = P = highly significant at 99% level

On both days the same 12 tasters were divided into panels of 6 and their scores were treated separately. In this case their rankings are given together. It appears that the smaller panel came to the same conclusions as the larger one. In most cases the level of significance is also the same. It should be noted, however, that we do not aim at reducing the number of panelists. On the contrary, depending on the parameters of the material to be tested, and beyond this, in order to limit as far as possible the drawbacks of subjective judgement, it is desirable to increase the panel. On the other hand, when fewer, but highly trained panelists are only available, replicated tests will give acceptable results.

2.2. Sensory analysis by means of difference tests

In the next part of our work we applied triangle and duo-trio tests to various sour cherry juice and mushroom paste samples.

2.2.1. Triangle test. This test was carried out on both types of samples by the same panel of tasters. The tasters performed always the difference test and did not have to answer the question "which is the better one?" of the quality test. In the case of sour cherry juice 6 triads were formed out of 4 samples. The test was repeated with the same samples 24 hours later. The results were subjected to significance tests by means of the tabular method. The results are shown in Table 5.

Tests performed on the first day showed a significant difference in all variations. Judgments passed on the next day showed however a somewhat different pattern, namely the control sample differed on a 99.9% level of probability not only from the sour cherry juice prepared with cold aroma concentrate, but also from the juice made with aroma concentrate prepared by a hot process. On the other hand, the panelists found no significant difference within the triads containing no control sample.

Next the scores given on the two days were treated jointly as the judgements of a single panel. In this way we hoped to gain information on the possible improvement of the efficiency of judgement in case of repeated testing by a small panel. It appeared that, like on the first day, in this case, too, there were significant differences within each sample comparison, and these were either of the same value or more marked than could be concluded from the judgements of the smaller panel.

In the triangle testing of mushroom pastes the four samples differing only in respect of mushroom content were compared in all variations, so that six triads were submitted to the panelists. Their judgements indicated significant differences, except between the two samples with the lowest mushroom contents. In the case of four triads all panelists gave the correct answer.

2.2.2. Duo-trio tests. The duo-trio test is a preferred form of difference testing. Compared to the triangle test it has the advantage of excluding the possibility of re-tasting and can be successfully used even when besides the tested properties there is also a detectable difference in some other factor which can be easily observed when serving one sample beside the other.

Since according to the literature this method is mainly applicable to comparison with control samples, using the control as a standard we prepared three groups of sour cherry samples. A panel of eight tasters was available for this test.

In case of mushroom pastes a panel of ten tasters performed the duo-trio test. The sample with the lowest mushroom content was chosen as standard. The results of the tests are given in Table 6.

Table 5

Significance test of the taste of sour cherry juice and mushroom paste samples judged by the triangle test

Date of evaluation: October 1969

Symbols of compared samples	Number of panelists	Number of correct judgements	Level of significance
	1st day		
Sour cherry juice			
1 : 2	12	10	***
1 : 3	12	9	**
1 : 4	12	8	*
2 : 3	12	8	*
2 : 4	12	9	**
3 : 4	12	8	*
	2nd day		
1 : 2	12	11	***
1 : 3	12	12	***
1 : 4	12	9	**
2 : 3	12	5	—
2 : 4	12	6	—
3 : 4	12	5	—
	overall results of the two days		
1 : 2	24	21	***
1 : 3	24	21	***
1 : 4	24	17	***
2 : 3	24	13	*
2 : 4	24	15	**
3 : 4	24	13	*
Mushroom paste			
1 : 2	12	6	—
1 : 3	12	12	***
1 : 4	12	12	***
2 : 3	12	10	***
2 : 4	12	12	***
3 : 4	12	12	***

The number of minimum correct judgements for definitely significant differences in the case of 12 panelists 8, 9 and 10, in case of 24 panelists 13, 14 and 16, in the increasing order of probability levels.

— = not significant, * = P = significant at 95% level,

** = P = significant at 99% level, *** = P = significant at 99.9% level

Symbols of samples of mushroom paste: 1 = paste base + 25 g of mushroom paste, 2 = paste base + 50 g of mushroom paste, 3 = paste base + 100 g of mushroom paste, 4 = paste base + 150 g of mushroom paste.

As seen from the Table, in the case of sour cherry the panelists indicated in all cases a significant difference at 95% level, while in the mushroom paste tests no significant difference was found by the panelists.

2.2.3. Comparison of the difference tests. In testing sour cherry juices the panelists detected by both methods significant differences within the groups

Table 6

Significance test of sour cherry juice and mushroom paste samples judged by duo-trio test

Date of testing: October 1969

Symbols of compared samples	Number of panelists	Number of correct judgements	Level of significance
	duo-trio test		
Sour sherry juice			
1 : 2	8	7	*
1 : 3	8	7	*
1 : 4	8	7	*
Mushroom paste			
1 : 2	10	5	—
1 : 3	10	5	—
1 : 4	10	8	—

The number of minimum correct judgements for definitely significant differences in the case of 8 panelists 7, 8 and 8, in the case of 10 judges 9, 10 and 10, in the increasing order of probability levels

— = not significant

* = significant at 95% level

of samples; however, while the duo-trio test indicated in all three cases a significance at 95% level, the triangle test showed within two triads a difference of higher significance level (Fig. 3).

In the mushroom paste tests no significant difference was found by the panelists using the duo-trio test, but by means of the triangle test a difference at 99.9% significance level was observed between the standard and the two samples whose compositions differed in a greater degree from the standard.

The difference between the results of the two methods may be attributed to the fact that in the case of mushroom pastes changes in mushroom content caused such marked alterations in colour which, when the samples were placed side by side, were clearly apparent to the panelists.

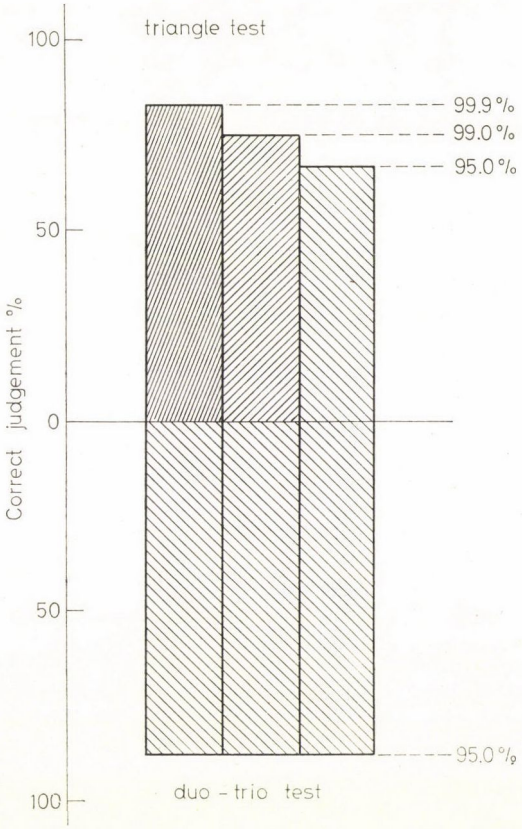

Figure 3. Comparison of the duo-trio and triangle tests of various sour cherry juice samples

3. Conclusions

When using the scoring test for the evaluation of the sensory quality the obtained scores — where the series of scored values are not independent — should be evaluated in addition to Kramer's method, by t test, and first of all by difference t test or otherwise by the comparison of the averages of paired data. The application of this last method of evaluation is certainly correct, because having a higher efficiency, the true differences between the sensory properties of the samples are expressed with greater sensitivity, more unambiguously and definitely. A further advantage of this method of calculation is that the assumption that the standard deviation of the two populations is equal, becomes irrelevant. When comparing Kramer's calculations with the results of the t tests we find that with respect to the differences in taste, odour, etc. of the samples, the first method of evaluation provides less information than the second.

In order to ensure optimum conditions for sensory tests it is necessary to set up a panel of sufficiently trained and experienced tasters of high sensitivity and not randomly chosen, since the relative homogeneity of judgements might be one step in the direction of the improved accuracy of test procedures and of a more realistic utilization of their results. Further, it might become necessary to repeat the test, particularly when a small panel performs the work.

According to the experience gained with sensory evaluation by means of difference tests, triangle and duo-trio tests should be given a greater role in Hungary, since these tests by their very nature greatly limit the subjective factors. In cases when the material under investigation might display in addition to the investigated parameter noticeable difference in some other property as well, the duo-trio test should be given absolute preference over the triangle test.

*

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THE EFFECT OF RADURIZING RADIATION DOSES ON LOW DENSITY POLYETHYLENE FILMS

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The experiments aimed at establishing any changes important from the packaging aspect, caused by ionizing radiation in the packaging material when food packed into low density polyethylene film is subjected to radurization. To clarify this question 0.004 cm thick low density polyethylene films were treated with radurizing doses of 10 to 800 krad and changes caused by ionizing radiation in the optical density and mechanical properties of the film were recorded.

In the optical density tests changes in ultra-violet, visible and infra-red radiation absorption were determined. From the changes in light absorption in the infra-red range changes in the structure of the polyethylene film could be studied as a function of the radiation dose.

The experiments have shown a change in the infra-red absorption of the polyethylene film after treatment with 100 krad (Fig. 1 and Tables 1—3) indicating a change in the structure of the polymer.

Absorption diagrams plotted in the visible (Fig. 2) and ultra-violet range (Fig. 3) confirmed the fact that radurization, that is doses below 1 Mrad have no influence on the absorption properties of the material.

Of the mechanical properties, tensile strength and elongation at break were tested, as being the most important mechanical parameters from the point of view of packaging technology.

The mechanical tests in the machine direction have shown a decrease in the tensile strength and elongation at break of low density polyethylene films (Tables 5—6 and 8—9, respectively) with increasing radiation doses, while there were practically no changes in these parameters in the transverse direction.

Summing up it may be stated that radurizing radiation doses cause no change in the tested low density polyethylene film which would render the latter unsuitable for the packing of foodstuffs.

Intensive research with respect to the applicability of ionizing radiation in the food industry has necessitated the examination of the effect of radiation on various plastic based packaging films, since ionizing radiation may cause structural changes, fission, rearrangement and formation of chemical bonds in the polymer film. As a result the physical, chemical, microbiological and mechanical properties of the plastic may suffer some detrimental change.

A close relationship exists between the properties and structures of the various polymer films. Much work has been done to detect and describe these correlations of which the work of HOLZMÜLLER and ALTENBURG (1961), WOLF (1962) and HOUWINK and STAVERMAN (1963) shall be mentioned here as discussing in detail and from many aspects these correlations. It appears from these correlations that all investigations of solid polymer films have to start from the structural characteristics of the material.

Accordingly, when studying the changes caused by ionizing radiation in the properties of low density polyethylene films used widely for the packing of food, changes in the structure of the material were also recorded, since an adequate answer to the question of the applicability of the material can only be expected from the concomitant consideration of all these observations.

It was the aim of our research to determine the changes caused by ionizing radiation in low density polyethylene films used for packaging foodstuffs, or, more accurately to determine the changes caused by radurizing (food pasteurizing) doses in the properties of these packaging materials.

1. Materials and methods

1.1. Description of the membrane

In the tests a 0.004 cm thick film, produced from imported low density (0.91 g per cubic cm) polyethylene granulate by the Plastic Processing Works Hungaria (Hungária Műanyagfeldolgozó Vállalat) and complying with the specifications for food packing materials, was used.

1.2. Description of the test methods

1.2.1. Irradiation. The low density polyethylene film was radiation treated with the ^{60}Co gamma-radiation source of 50 000 Ci rated activity of the Central Food Research Institute. The doses were adjusted between 10 and 800 krad according to the requirements of the test. Temperature during irradiation varied beween 10° and 15° C, the dose rate of the radiation source was 100 krad per hour.

The absorbed doses were determined by means of chemical dosimetry using the method of WEISS et al. (1955).

The irradiated samples were stored one on the top of the other, wrapped into paper, at room temperature (about 25 °C) and at about 65% relative humidity.

1.2.2. Infra-red spectrophotometry. IR spectrophotometry is an often and successfully applied method in the study of the structure of plastics. Hence, there is an extensive literature dealing with the preparation and evaluation of IR spectra, of which the work of DAVIES (1963) and VARSÁNYI (1963) shall be mentioned, as having been of the greatest help in our work. The study of the changes of practical implications in the structure of the irradiated polyethylene film was therefore performed primarily on the basis of the IR spectra.

The effects of ionizing radiation on plastics were studied by us from the works of BERSCH et al. (1959), ADLER (1963), SCHMIDT-LORENZ and GRÜNWALD (1963) and PROKOSCH (1968) which discuss in general the changes caused in polymers by radiation effects

We investigated the changes caused by ionizing radiation in polyethylene films in consideration of the works of BENT (1957), LANZA (1957), HARPER (1959), LEVY (1961), SALOVEY (1962), BIXLER et al. (1963), LUONGO and SALOVEY (1963), MARTIN and GRIESACKER (1963), SEARS (1964), ODIAN and BERNSTEIN (1964) and of AUERBACH (1967). It must be, however, pointed out that these authors did not study the effect of radurizing, as is foodstuff pasteurizing or below 1 Mrad radiation doses, but of considerably higher doses, so that we obtained from their work not so much data as certain points of view.

Neither did the works dealing with the irradiation of packaged foods and with the changes due to irradiation offer any help with respect to the changes to be expected in the irradiated polyethylene film, since works, such as those published by MÜNZEL (1968), KEAY (1968), CHAMPAGNE and NAWAR (1969), MEHRING (1969) discuss in detail only changes in the irradiated foodstuffs.

A concise picture of the requirements which the packaging materials have to meet in general is given by VARSÁNYI (1969), while BEDNARCZYK (1968) deals with the pertaining hygienic specifications. These works devote, however, no special attention to irradiated polyethylene as a packing materia used in the food industry.

Thus, in choosing the method of measurement and of the evaluation of the results and also the aspects of testing we were able in our work to rely on the literature.

Film specimens cut to fit the instrument and having a thoroughly cleaned surface were used for the tests which were carried out by means of the recording infra-red spectrophotometer type UNICAM SP 200 at an energy level marked ½ E, at room temperature and normal humidity. The medium for comparison was air. The spectra were recorded at 100, 200, 400 and 800 krad dose levels, each measurement being repeated five times.

1.2.3. Recording of ultra-violet and visible spectra. Changes in the ultraviolet transmittance, that is in the absorption properties of low density polyethylene films and in their visible light transmittance which affects appearance, were determined photometrically by means of the PERKIN—ELMER UV 137 visible and ultra-violet spectrophotometer at 100, 200, 400 and 800 krad dose levels, using five samples at each dose level.

1.2.4. Tensile strength and elongation at break tests. Tensile strength and elongation at break of low density polyethylene film samples were measured in machine direction and transversally on 30 mm wide and 200 mm long specimens using 10 samples at each dose level. The tests were carried out on FMPW 500 type tensile test machine of VEB Werkstoff Prüfmaschinen in accordance with the Hungarian Standards MSZ 5360—52 and MSZ 5355—51. The set limit on the machine was 100 kp, the accuracy of readings in case of tensile strength tests was 0.2 kp, in case of elongation at break tests 0.5%.

1.2.5. Statistical evaluation of the results. The experimental results were evaluated by mathematical statistical methods (SVÁB, 1967). With respect to values conspicuously deviating from the others the series of data were first checked by calculating Dixon's r criterion and excessive values were excluded from further calculations. Next the homogeneity of deviations was established by means of Bartlett test and variance analysis was used to determine any eventual effect of ionizing radiation on the investigated properties of the polyethylene film. Comparison of the data of the radiation treated film with those of the untreated film was performed by means of Student test.

2. Results

2.1. Evaluation of IR spectrophotometric data

Evaluation of the IR spectrophotometric data of various polymers is being dealt with in detail in the literature. Since polyethylene is one of the most important plastics its structural properties have been studied with the help of IR spectrophotometry by many investigators. Of these SCHNELL (1954), KRIMM et al. (1956), WOOD and LUONGO (1961), DAVIES (1963) and ZBINDEN (1964) shall be mentioned here whose work was of great help in our investigations. We studied the changes in the proportion of amorphous and crystalline regions on the basis of the work of NICHOLS (1954), HENDUS and SCHNELL (1961), KOCSKINA (1968) and DIETEL (1969) of the ample literature on the crystal structure of polyethylene. In order to determine the radiation effects we have also considered bands which showed intensity changes as function of the dose. We have thus recorded changes in the intensities of the absorption bands pertaining to 725, 1020, 1299, 1370, 1475, 1740, 1887 and 2950 cm^{-1} wavenumber values vs. the applied radiation dose.

Changes in the proportion of the amorphous and crystalline regions were determined from the changes in the intensities of absorption bands pertaining to 725, 1299 and 1887 cm^{-1} wavenumbers.

Changes in the number of the $—CH=CH_2$ unsaturated molecular bonds were determined from the changes in the absorption degree of the bond pertaining to the 1020 cm^{-1} wavenumber. The various physical properties of the polymer — such as molecular weight, viscosity, etc. — depend on the number of the $—CH_3$ terminal groups and of the —CH— bonds. For this reason the changes in the intensity of the absorption band at 1370 cm^{-1} wavenumber, which is characteristic of the $—CH_3$ terminal group, as well as changes at 1475 and 2950 cm^{-1} in the intensity of the absorption bands characteristic of the number of —CH— bonds, were also recorded.

The wavenumber range between 1650 and 1785 cm^{-1} provides information on the number of the various —C=O bonds, and in this range the

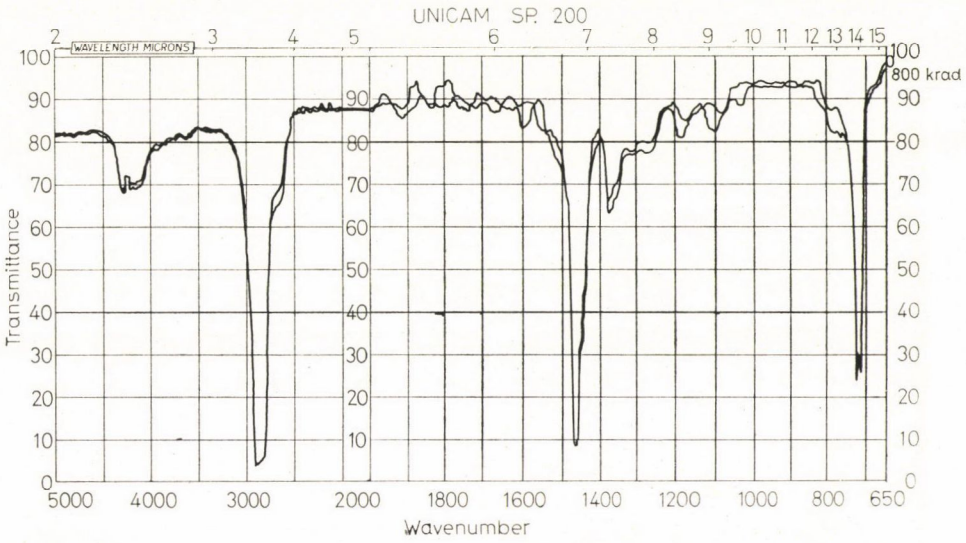

Figure 1. IR absorption spectrum of an untreated and a 800 krad dose radiation treated 0.004 mm thick polyethylene film. UNICAM SP 200 spectrophotometer, against air. There was a 241-day interval between irradiation and measurement

Table 1

The effect of ionizing radiation on the IR transmittance of a 0.004 cm thick polyethylene film

(Comparison of the transmittance values pertaining to some characteristic wavenumbers of the spectra obtained by means of the Unicam Sp 200 spectrophotometer)

Wave-number cm^{-1}	Percentage transmittance ($\bar{x} \pm s$) at				
	0	100	200	400	800
	krad doses				
725	29.0±1.83	27.3±1.52	26.2±3.22	27.7±3.20	31.3±1.53
1020	93.0±1.52	96.1±1.89	95.9±1.69	95.6±1.99	96.3±1.78
1293	83.5±3.45	77.6±2.43	78.8±3.99	83.3±2.71	79.5±3.05
1370	69.1±3.62	66.3±1.62	66.1±2.95	69.2±2.94	69.8±2.68
1475	13.0±2.12	10.1±1.33	10.9±2.45	11.2±1.36	11.9±1.55
1740	92.7±1.74	94.8±3.59	93.1±3.02	94.8±1.31	93.0±4.18
1887	94.5±1.33	92.4±3.58	93.3±3.58	94.5±1.52	91.1±1.83
2950	8.1±1.60	6.1±1.75	6.5±1.71	4.8±1.16	6.7±1.41

$\bar{x}$ = mean of five measurements
s = standard deviation

The values underlined with a single line represent transmittances significantly higher than the transmittance of the untreated sample for the same wavenumber.

The values underlined by a broken line represent transmittances significantly lower than that of the untreated sample.

Table 2

Bartlett's test for homogeneity of variances with data of Table 1

Degrees of freedom	Σ FG	= 156
Pooled estimate of variance	S_e^2	= 2.0668
	C	= 1.0883
	$\chi^2_{calc.}$	= 39.99
	$\chi^2_{95\%}$	= 54.62
	$\chi^2_{calc.}$	$< \chi^2_{95\%}$

The differences between variances are not significant.

intensity of the absorption band at 1740 cm^{-1} indicates the changes in the numerical proportion of ester type bonds.

The IR absorption spectra of non-irradiated and with 800 krad dose irradiated polyethylene films are shown in Fig. 1.

The mean value ($\bar{x}$) of the results of measurements performed as functions of the chosen wavenumber and dose level and the estimated deviations (s) are summed up in Table 1.

The homogeneity of the deviations was checked by means of the Bartlett test (Table 2) and since these failed to show a significant difference at $P = 95\%$ significance level the data of Table 1 were subjected to variance analysis whose results are shown in Table 3.

Variance analysis showed that ionizing radiation significantly altered the IR transmittance of low density polyethylene.

Table 3

Analysis of variance of yields from Table 1

Source of variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Total	264 853.60	195		
Wavenumber	263 345.07	7	37 620.73	35 159.56***
Dose	385.98	4	96.49	90.18***
Interaction	19.41	28	0.69	0.65
Residual	1 103.14	156	1.07	

$F_{99.9\%}$ (7/156) = 3.97***
$F_{99.9\%}$ (4/156) = 4.62***
$F_{99.9\%}$ (28/156) = 2.21***
$F_{95\%}$ (28/156) = 1.56*

The least significant difference between the mean of any two combinations is:

$$SD_{95\%} = 1.293$$

2.2. Evaluation of visible and UV spectra

Spectra in the visible wavelength range (350—750 nm) record changes in appearance caused by radiation (SCHMIDT-LORENZ & GRÜNEWALD, 1963, 1964), while spectra in the UV range (190 to 390 nm) provides more information on structural changes in the material (LEVY, 1961; HOLZMÜLLER & ALTENBURG, 1961; KLINE, 1963; SCHMIDT-LORENZ & GRÜNEWALD, 1963 and 1964).

While spectra in the visible range indicate changes in the appearance of the polymer film (e.g. discolouration, impaired transparency, etc.), the UV spectra record the anisotropic changes in the energy absorption of electrons responsible for the molecular bonds. These anisotropic changes are caused by changes in the number of double bonds, particularly of conjugated double bonds.

In the case of polymers these methods provide obviously fewer and less detailed information than IR spectroscopy, and accordingly there has appeared less in the literature on this branch of spectroscopy.

The visible spectra of non-irradiated and with 800 krad radiation treated low density polyethylene films are shown in Fig. 2, the UV spectra of similar films is presented in Fig. 3.

It appears from the Figures that irradiation causes no significant differences either in the visible or in the ultra-violet spectra, that is in the absorption properties of films treated with 800 krad as compared to the spectra of the untreated samples.

2.3. Effect of ionizing radiation on tensile strength and elongation at break

Tests evaluating changes in the mechanical properties of plastic films exposed to ionizing radiation are treated concisely among others in the works of HOLZMÜLLER and ALTENBURG (1961), WOLF (1962), CHAPIRO (1962) and HOUWINK and STAVERMAN (1963). HARPER (1959), ADLER (1963), MARTIN and GRIESACKER (1963), ODIAN and BERNSTEIN (1964) discuss in particular the changes taking place in radiation treated polyethylene. These authors, just as others cited by them, investigated, however, the effect of radiation doses of the order of some Mrad, while they were not concerned with doses below 1 Mrad. For this very reason we intended to use the comparison possibilities of mathematical-statistical methods to a greater degree and to base our conclusions on the results of the latter.

The results of tensile strength tests performed on polyethylene films which have been irradiated with 10, 20, 40, 100, 200, 400 and 800 krad doses are given in Table 4.

The experimental data were checked for values deviating from the others by means of Dixon's r criterion and the extremes were excluded from further

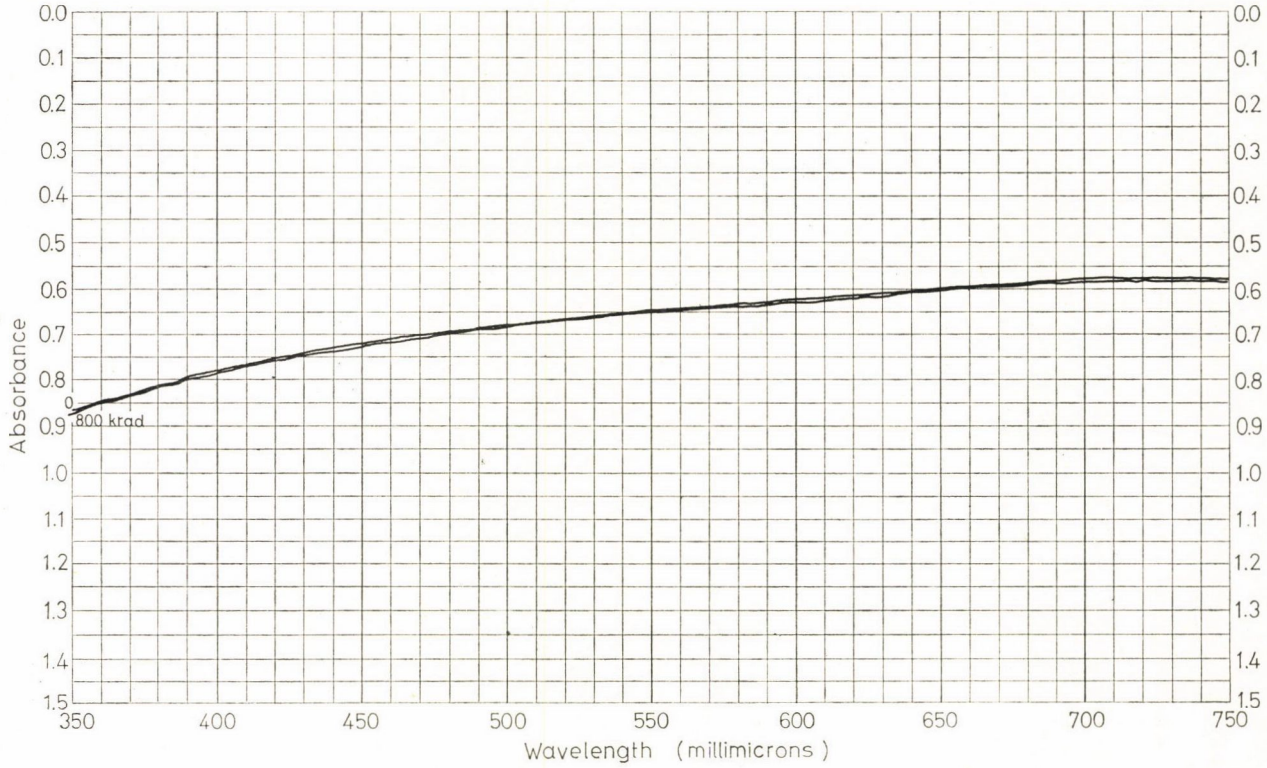

Figure 2. Visible light absorption spectrum of an untreated and an 800 krad radiation dose treated 0.004 cm thick polyethylene film. PERKIN-ELMER UV 137 type spectrophotometer against air.
There was an interval of 243 days between irradiation and measurement

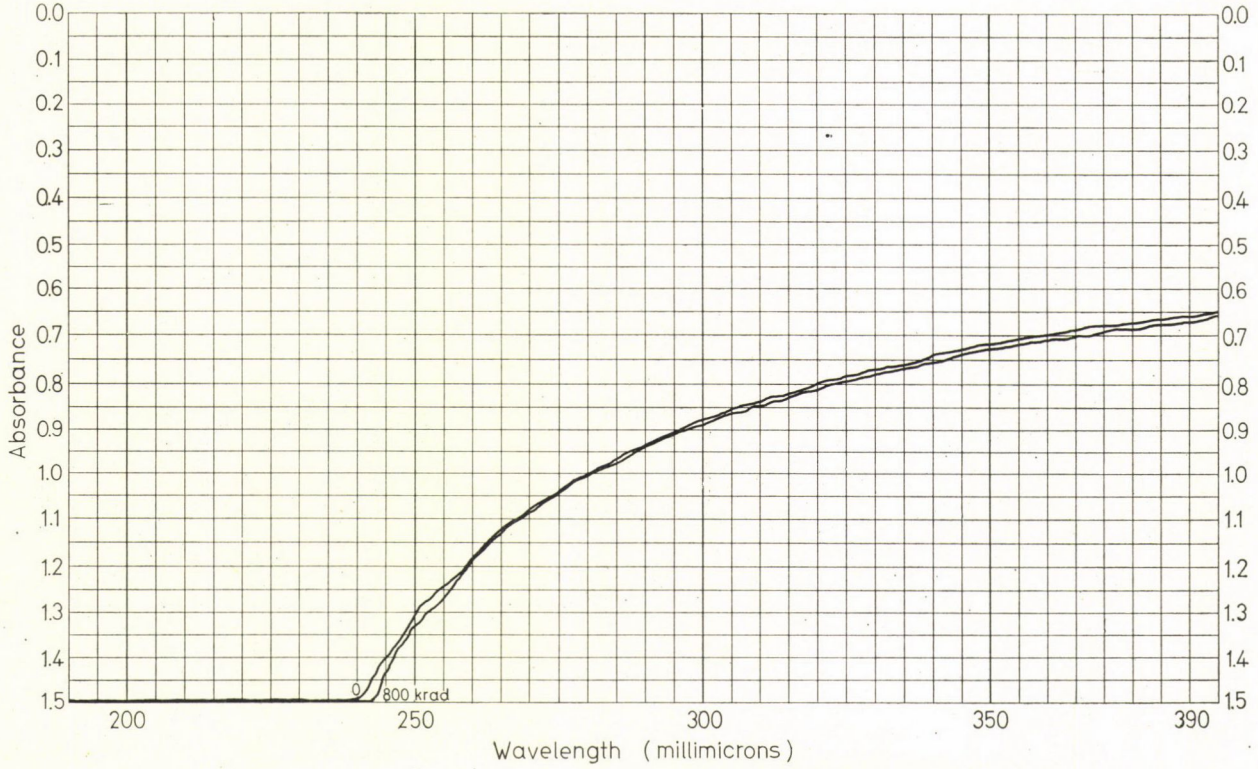

Figure 3. UV absorption spectrum of an untreated and an 800 krad radiation dose treated 0.004 cm thick polyethylene film. PERKIN-ELMER UV 137 type spectrophotometer, against air. There was an interval of 242 days between irradiation and measurement

Table 4

The effect of ionizing radiation on the tensile strength of a 0.004 cm thick polyethylene film

Testing of 100×10 mm specimens of polyethylene film by means of a VEB Werkstoff Prüfmaschinen (Leipzig) tensile strength tester applying an elongation rate of 30 mm/min and a 50 mm changing distance. The measurements were carried out at room temperature and relative humidity

Tensile strength (kp/cm²) after irradiation with															
0		10		20		40		100		200		400		800	
krad															
M	T	M	T	M	T	M	T	M	T	M	T	M	T	M	T
118	163	96	148	104	155	104	155	111	141	104	155	126	163	104	148
126	178	96	163	96	155	104	155	111	163	111	148	141	163	96	148
118	178	96	192	96	170	104	178	111	163	104	163	133	141	96	148
104	163	96	155	104	185	88	163	96	170	104	170	148	141	96	170
118	163	96	163	104	170	88	178	96	170	104	200	141	141	96	178
104	163	96	141	96	141	88	148	96	155	104	163	141	148	96	133
104	148	118	155	104	178	88	178	104	141	104	170	104	163	104	163
111	163	126	192	96	163	88	178	104	148	104	163	178	170	104	178
104	163	118	141	96	141	88	178	96	141	104	163	104	141	104	178
104	163	96	170	96	178	88	155	88	163	104	170	133	178	96	170

M = machine direction
T = transverse direction

The values underlined by broken line in the columns were found to be extreme in calculations for Dixon's r-criterion and were excluded from further calculations.

There was an interval of 14 days between irradiation and measurement.

calculations. In Table 4 the data considered extreme are underlined with a dashed line; these have not been used in further calculations.

The homogeneity of deviations was tested with the help of the Bartlett test and since the result of calculation showed no significant difference the effect of radiation treatment was now investigated by variance analysis. Following variance analysis the untreated and radiation treated films were compared by using Student's t test.

The results of calculations performed with the data of tensile strength tests in machine direction are presented in Table 5, while the results of calculations performed with the data of tensile strength tests in transverse direction are given in Table 6.

The results of these calculations have shown a significant change in the tensile strength in machine direction of the polyethylene film irradiated with doses as low as 40 krad, while no difference was observed in the tensile strength of the same film in transverse direction.

Table 5

Analysis of variance of tensile test data obtained in machine direction (Table 4)

Radiation dose krad	n	$\bar{X}$	$S_{\bar{x}}$
0	10	111.1	7.85
10	10	103.4	11.49
20	10	99.2	3.92
40	10	92.8	7.33
100	10	101.3	7.66
200	9	104.0	0
400	10	134.9	20.34
800	10	99.2	3.92

Calculated by Bartlett test $\chi^2 = 64.216$
from the Table $\chi^2_{99.9\%} = 24.3$

Variances show a highly significant deviation.

According to the results of the χ^2 test among dose levels the variances pertaining to 20, 200 and 800 krad, respectively, are significantly lower, the variance pertaining to 400 krad is significantly higher than the rest.

Results of the F test, without accounting for the data pertaining to 20, 200, 400 and 800 krad, respectively, are:

Source of variation	SQ	FG	MQ	F
Between groups	1698.1	$k-1 = 3$	566.03	6.66**
Within groups	3061.0	$n-k = 36$	85.03	($F_{99.9\%} = 6.82$)
Total	4760.0	$n-1 = 39$		

The difference between mean values is highly significant.

Result of the t test:

Comparison of the mean pertaining to 0 krad and of the mean values of irradiated films.

Dose, krad	t
10	1.66
20	4.06***
40	5.11***
100	2.70*
200	2.71*
400	3.27**
800	4.06***

where

* P $\leqq$ 95% (2.101)
** P $\leqq$ 99% (2.878)
*** P $\leqq$ 99.9% (3.922)

Table 6

Analysis of variance of tensile strength data obtained in transverse direction (Table 4)

Radiation dose krad	n	$\bar{X}$	$S_{\bar{x}}$
0	10	164.5	8.08
10	10	162.0	17.44
20	10	163.6	14.58
40	10	166.6	11.88
100	10	155.5	11.30
200	9	162.8	6.96
400	10	154.9	13.34
800	10	161.4	15.24

Calculated by Bartlett test $\chi^2 = 9.38$ from the Table $\chi^2_{99.9\%} = 24.3$

There is no significant difference between variances.

Result of the F test:

Source of Variation	SQ	FG	MQ	F
Between groups	1 206.14	k−1 = 7	172.3	1.066
Within groups	13 044.90	n−k = 71	183.7	($F_{95\%} = 2.18$)
Total	14 250.97	n−1 = 78		

There is no significant difference between the mean values.

Table 7

Effect of ionizing radiation on the elongation at break of a 0.004 cm thick polyethylene film

(Experimental conditions the same as given in Table 4)

Elongation at break (%) after irradiation with															
0		10		20		40		100		200		400		800	
krad															
M	T	M	T	M	T	M	T	M	T	M	T	M	T	M	T
360	172	92	200	170	142	126	150	230	134	354	104	194	142	286	182
314	186	100	206	166	190	166	206	320	154	262	148	234	166	266	184
282	218	130	180	142	158	110	170	290	152	262	90	214	130	294	140
296	150	129	172	146	204	88	194	294	140	306	112	240	180	300	154
230	124	96	216	134	188	212	184	264	180	284	190	452	130	232	170
326	158	116	150	200	160	132	150	300	158	280	160	266	230	282	130
290	176	130	172	198	184	120	160	286	151	302	172	240	134	314	156
322	144	221	164	160	172	214	176	266	166	238	121	220	210	320	152
284	154	144	194	120	130	122	148	288	178	252	140	200	216	250	178
336	162	146	146	180	134	120	166	286	196	202	120	212	156	298	166

M = machine direction T = transverse direction

The figures underlined with a dashed line were found to be extreme by means of Dixon's *r* criterion and were therefore excluded from further calculations.

There was an interval of 14 days between irradiation and measurement.

Table 8

Analysis of variance of elongation at break data obtained in machine direction (Table 4)

Radiation dose krad	n	$\bar{X}$	$S_{\bar{x}}$
10	10	304.0	34.19
20	7	127.9	14.72
40	10	161.6	25.20
100	10	141.0	40.39
200	10	282.4	23.08
200	10	274.2	39.49
400	9	202.0	21.37
800	10	284.2	26.34

Calculated by Bartlett test $\chi^2 = 20.98$
from the Table $\chi^2_{99.9\%} = 24.3$

There is no significant difference between variances.

Results of the F test:

Source of variation	SQ	FG	MQ	F
Between groups	330 327.81	k−1 = 7	47 189.69	47.30***
Within groups	67 841.34	n−k = 68	997.67	($F_{99.9\%} = 4.13$)
Total	398 169.14	n−1 = 75		

There is a highly significant difference between the mean values.

Result of t test:

Comparison of the mean pertaining to 0 krad and of the mean values of irradiated films:

Dose, krad	t
10	12.07***
20	10.06***
40	22.74***
100	2.57*
200	4.52***
400	10.54***
800	2.74*

where

*	$P \leq 95\%$	(2.101)
**	$P \leq 99\%$	(2.878)
***	$P \leq 99.9\%$	(3.922)

Table 9

Analysis of variance of elongation at break data obtained in transverse direction (Table 7)

Radiation dose krad	n	$\overline{X}$	$S_{\bar{x}}$
0	10	164.4	24.38
10	10	180.0	22.32
20	10	166.2	24.21
40	10	170.4	18.72
100	10	160.9	18.19
200	10	135.7	30.24
400	10	169.4	35.92
800	10	161.2	17.09

Calculated by Bartlett test $\chi^2 = 8.45$
from the Table $\chi^2_{99.9\%} = 24.3$

There is no significant difference between variances.

Results of the F test:

Source of variaton	SQ	FG	MQ	F
Between groups	11 476.55	k−1 = 7	1639.5	2.42*
Within groups	48 591.4	n−k = 72	674.88	($F_{95\%} = 2.13$)
Total	60 067.95	n−1 = 79	760.35	($F_{99\%} = 2.95$)

The difference between mean values is significant.

Results of the t test:

Comparison of the mean pertaining to 0 krad and of the mean values of irradiated films:

Dose, krad	t
10	1.36
20	0.16
40	0.58
100	0.35
200	2.24*
400	0.35
800	0.32

where

* $P \leq 95\%$ (2.101)
** $P \leq 99\%$ (2.878)

The elongation at break data of polyethylene films irradiated with doses of 10, 20, 40, 100, 200, 400 and 800 krad, respectively, are given in Table 7. Statistical evaluations performed by the same method as applied to tensile strength tests are given in Table 8 for measurements in machine direction and in Table 9 for measurements in transverse direction.

The results of calculations presented in Tables 8 and 9 indicate a significant change in the elongation at break of the irradiated polyethylene film in machine direction even in the case of radiation doses as low as 10 krad, while the elongation at break values in transverse direction showed almost no change.

3. Conclusions

Low density polyethylene films, suitable as packaging materials for foodstuffs, were subjected to various tests in order to determine changes, particularly changes important from the aspect of packaging technology, caused by ionizing radiation below 1 Mrad, that is by radurization doses.

The phenomena were interpreted, and conclusions drawn with the help of mathematical statistical methods. The homogeneity of deviations was determined by Bartlett test whereby information was gained on the comparability of experimental data. Two-variate and one-variate variance analysis including Student's t test was used to compare the properties of untreated films with those treated with different radiation doses in order to demonstrate the significant effect of a certain radiation dose.

Changes in the structure of the material were studied with the help of IR spectroscopy.

The infra-red spectrum of low density polyethylene film presented in Fig. 1 shows a significant difference with respect to infra-red radiation transmittance between the untreated sample and the eight samples irradiated with 100 krad, six samples irradiated with 200 krad and five samples irradiated with 400 and 800 krad, respectively, at the eight wavelengths whose data have been subjected to analysis and presented in Table 1.

According to the data of Table 2 there is no significant difference between the deviations, so that variance analysis can be applied. The results of the latter are presented in Table 3 and show that light absorption depends to a very highly significant degree on the wavelength and further that radiation treatment influences very significantly the infra-red absorption of the polyethylene film. Interaction between wavenumber and dose is not significant, indicating a generally uniform change in the infra-red absorption of the investigated polyethylene film as radiation progresses.

Investigation of the changes of the absorption values pertaining to the chosen wavenumbers of the infra-red spectrum (Table 1) reveals that at the band pertaining to the wavenumber 1020 cm^{-1} all doses cause a significant drop in absorption.

As a result of irradiation with 100 and 200 krad doses the absorption of the band at 1370 cm^{-1} wavenumber increased significantly, the absorption values at 1475 and 2950 cm^{-1} showed a significant increase at all radiation doses, that is at 100, 200 and 400 krad.

At the absorption band of 725 cm^{-1} the doses of 100, 200 and 400 krad caused significant increase and 800 krad a significant decrease in absorption.

The intensity of the absorption band at 1299 cm^{-1} wavenumber increased significantly as a result of irradiation with 100, 200 and 800 krad doses, while the intensity of the absorption band at 1887 cm^{-1} wavenumber increased significantly after irradiation with 100 and 800 krad doses.

The intensity of the absorption band at 1740 cm^{-1} wavenumber decreased significantly as an effect of irradiation with 100 and 400 krad doses.

Visible and UV spectra (Figs 2 and 3) suggest that radurizing doses cause no significant change in the appearance of the film or in its UV absorption.

Investigation of the changes caused by irradiation in the mechanical properties, that is mainly in the tensile strength and elongation at break of the film, show no statistically detectable change in these properties.

Tensile strength in machine direction of the investigated low density polyethylene film (Table 5) shows a significant difference at certain doses compared to the tensile strength of untreated samples, but this difference is not quite consistent. If the average values of tensile strength are reduced, that is deviation is increased, no radiation effect will be noticeable.

It appears from the calculated values in Table 6 that radiation treatment caused no significant difference in tensile strength in transverse direction.

The results of calculations presented in Table 8 indicate a significant difference in elongation at break in machine direction as a result of radiation treatment, that is elongation at break decreased in dependence on the dose without, however, displaying a consistent change.

Variance analysis data of the elongation at break data in transverse direction (Table 7) are given in Table 9 and show that only the dose of 200 krad has caused a significant difference compared to the untreated film. This dose reduced elongation at break by 20%, but if the deviations are accounted for, the effect of radiation cannot be detected.

It appears from the above that radurizing doses change significantly the IR absorption of the irradiated low density polyethylene film, but cause no change in UV and visible light absorption. Radurizing doses reduce the tensile strength and elongation at break of the irradiated film in machine direction, while the same parameters in transverse direction remain practically unchanged after the same treatment.

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NEW APPARATUS TO STUDY THE PRESSING PROCESS, EXPERIMENTAL AND EVALUATION METHODS, CORRELATION BETWEEN PRESSURE AND EQUILIBRIUM JUICE YIELD IN THE CASE OF APPLE PULP

I. KÖRMENDY

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A new apparatus suitable for the pressing of fruit and vegetable pulps and for investigating the pressing process is described. The maximum applicable pressure is 30 kp/cm^2 gauge pressure. The compression of the material and the weight of the juice pressed from the pulp are measured simultaneously. The scales of the instruments used for the measurements are photographed collectively at given time intervals. The corrections applied in the evaluation of the results are discussed. These are: juice absorption by the filter cloth and by the supporting elements, evaporation, conversion constant of the displacement meter, or, in other words, the displacement needed to remove the air from the pulp, and the friction of the piston. According to this method the data of the displacement meter must be converted to juice yield values (weight of juice). The following supplementary measurements must be carried out: Determination of the specific gravity of the juice, of soluble solids content, of fibre content and of viscosity of the juice.

The author reports on the results of pressing experiments with apple pulp, and also on a new method by means of which a correlation has been established between pressure and equilibrium juice yield using mathematical statistical methods and a table computer.

The determination of the equilibrium juice yield was based upon the assumption that after a sufficiently long pressing time the difference between the juice yield and the equilibrium juice yield is a negative exponential function of pressing time.

In 1970 a new apparatus for the investigation of the pressing process was put into operation. The apparatus was suitable for pressing juice out of fruit and vegetable pulps on laboratory scale. Its construction and the experiments carried out with it are a direct continuation of the research performed in this field in recent years.

Quoting only the most important from earlier and already published works, we wish to draw attention to the following facts: The fundamental principles of the pressing operation have been systematized by KÖRMENDY (1968) who summed up the results achieved in various fields, such as soil mechanics, filtration theory and food technology and generalized these results for three dimensional cases. His terminology is used in the present paper. In other pressing experiments the influence of the initial layer thickness of the pressed material was investigated and so was the possibility of characterizing fruit pulps from the aspect of pressing (KÖRMENDY, 1961, 1964).

The need for the construction of a new apparatus to investigate pressing arose, because the earlier method proved to be inadequate for the determin-

ation of all the physical factors involved in the pressing operation, further it was desirable to improve the accuracy of the earlier methods. Since the differential equation of the pressing of a layer of constant initial thickness is formally the same as the corresponding differential equation of diffusion many results of the diffusion theory have been adopted (CRANK, 1967).

The first aim with respect to the experimental pressing apparatus was to find the best method for the correction of the values indicated by the instruments and to convert the values thus obtained into the most expedient physical parameters. A further aim of the work was the elaboration of a method for the determination of the relationship between the applied pressure and the pertaining equilibrium juice yield, or of the equilibrium compression which is equivalent to the former.

1. Materials and methods

1.1. Apparatus for the investigation of pressing

The schematic diagram of the apparatus is shown in Fig. 1.

The pulp to be pressed is packed into a cylindrical chamber (1) which is closed on one side by a piston (2), on the other by a filter cloth and an appropriate supporting plate (3). The pulp is laterally surrounded by the cylindrical mantle (4) jacketed to provide constant temperature (5). The jacket is connected through inlet and outlet stubs and flexible pipes (6—7) into the water circulation of an ultrathermostat. The pressed juice flows into a receiver (8) on a balance. During pressing the piston is under air or carbon dioxide pressure which is led into the space above the piston through an appropriate pipe-line (9). This space can be put, if required, under vacuum through the pipe-line applied to this purpose (10) in order to move the piston in opposite direction to pressing. The two sides of the piston are hermetically separated by rubber sealing rings. The displacement of the piston is indicated by a displacement meter (11) of 1/10 mm scale division. The displacement meter contacts a rod (12) attached to the piston. The receiver is placed on the pan of a letter balance (13) of 5 kg maximum load, having a scale division of 1 gramme and 50 gramme scale end value. The weight of the juice can be read off the balance directly. The pressure applied for pressing is measured by a Bourdon gauge, having a 150 mm diameter casing, 0 to 25 kp/cm^2 scale with 0.2 kp/cm^2 divisions and an accuracy class 1.

The so-called pressing head, consisting essentially of the cylinder, the piston, the filter cloth and the supporting plate, including the necessary flanges, can be removed from its position above the balance with the help of a vertical column (15), and can be turned upside-down round a horizontal shaft (16) to be filled up with pulp and to remove the compressed cake.

The chamber containing the fruit pulp has a diameter of 80 mm and its height can be varied up to 80 mm.

The filter cloth is made of terylene, it is a double twill woven fabric with 9 warps and 9 wefts per cm^2.

Pressure is provided by a pressure tank, filled with air or carbon dioxide of 80 to 100 kp/cm^2 gauge pressure. A pressure reducing valve serves to set the pressure required for pressing. The pressure applied in the experiments did not exceed 30 kp/cm^2 gauge pressure and could be adjusted to a constant value by means of the reduction valve.

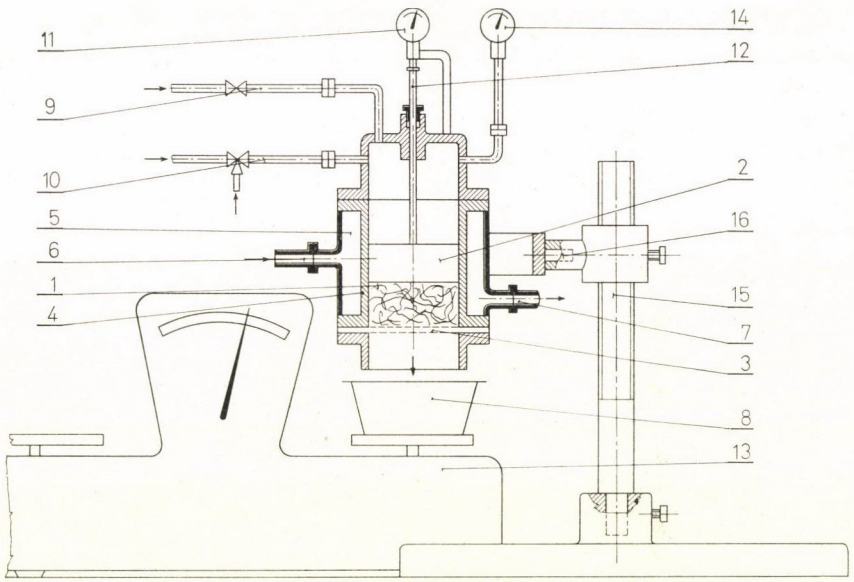

Figure 1. Schematic arrangement of the testing apparatus for pressing experiments, *1* chamber for the pulp; *2* piston; *3* filter cloth and supporting plate; *4* cylindrical mantle; *5* water jacket; *6* inlet pipe for thermostat water; *7* outlet pipe for thermostat water; *8* receiver; *9* inlet pipe for compressed air; *10* pipe line to vacuum pump; *11* displacement-meter; *12* rod attached to the piston; *13* balance; *14* manometer; *15* column; *16* horizontal shaft

Pressing time was measured with a chronometer of 0.2 sec scale division.

The relative humidity of the room in which the pressing experiments were carried out was measured by a Fischer type synthetic fibre hygrometer. The hygrometer had been previously calibrated with an Assmann psychrometer. The temperature of the room was measured with a mercury thermometer. During measurements the scales of the balance, the displacement meter, the pressure gauge and the chronometer were brought into the same line and the pointers photographed collectively. The photographs were made on 24×36 mm Fortepan film of 17 DIN sensibility, illumination time was 1/60 sec, with a diaphragm of 8 and light was provided by 2 Tungsraphot

S 250 W prestressed bulbs. The lamps were placed at a distance of 1 m, the camera at 95 cm from the scales of the instruments.

1.2. Raw materials and their storage

In the experiments large ripe Jonathan apples were used as raw material. The apples were stored whole, packed in sealed polyethylene bags in the refrigerator at temperatures between —1.5° and +3.0 °C, that is above the freezing point of apple. Storage temperature was checked by means of a maximum—minimum thermometer. The apples were put in the storeroom between the 19th and 28th of January, 1970. The material for the experiments was obtained by cutting segments, of 1/6th of an apple each containing part of the core. The batch made up of these segments was then ground.

1.3. Preparation of the pulp for pressing

The pulp was prepared by means of the meat mincer of the universal kitchen machine type Savaria EK—1000, manufactured in Hungary. The speed of the worm was 150 rpm, the diameter of the stationary perforated disk was 7.6 mm with 4.7 mm diameter perforations spaced at 10.9 mm. The perforations were situated at the vertices of equilateral triangles. The actual diameter of pressing the apple through the disk was 73 mm with four cutting blades. The external diameter of the grinding worm was 70 mm, the core diameter at the outlet side 44 mm. 0.1 % sodium benzoate was added to the pulp immediately after grinding.

1.4. Determination of the viscosity of the expressed juice

Viscosity was measured with a modified Ostwald viscometer having a capillary of 1.1 mm internal diameter and 73 mm length.

Water value at 25 °C was 31.6 sec, the volume of water pertaining to the water value was 3.5 cm^3. The Reynolds number at the water value was Re = = 143. Prior to measurement of viscosity the juice was filtered through a cotton wool pad placed into a funnel.

1.5. Determination of the soluble solids content, fibre content and specific gravity of the juice

Water soluble solids content was determined at 20 °C by means of an Abbe type refractometer which had been calibrated for sucrose. The determination of fibre content was carried out by centrifuging for 20 minutes at 6000 rpm in a centrifuge type LC—3 (manufactured by Zuglói Gépgyár, Budapest). The fibre free juice was decanted and the residual moist sediment weighed and expressed in the percentage of the initially centrifuged juice. The specific gravity of the juice was measured in a pycnometer at 25 °C.

1.6. Principle of the measurement; corrections

1.6.1. Theoretical model of the measurements and the corrections — The principle of measurement is illustrated in Fig. 2. The Figure shows the piston in its initial position and also in other positions during the pressing operation.

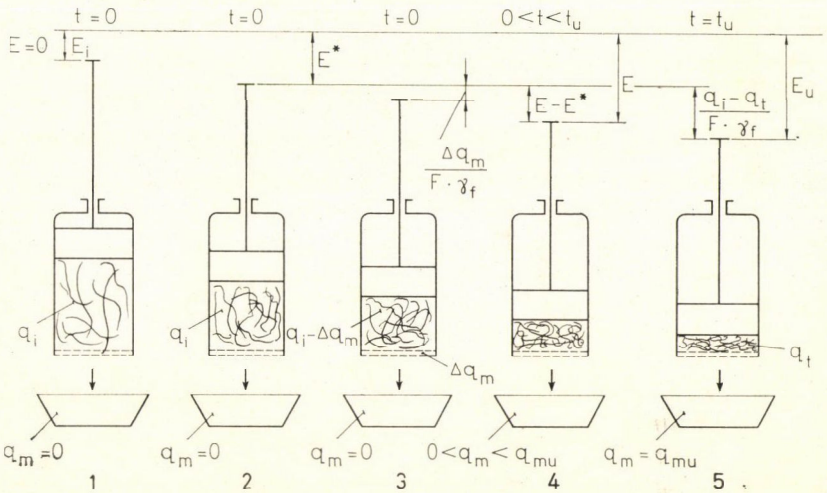

Figure 2. Principle of the measuring method and of the corrections. Position 1: initial state; position 2: state after the removal of air from the pulp; position 3: state after the absorption of juice by the filter cloth and supporting elements; position 4: state during measurement; position 5: final state of the measurement

The symbols used in the calculation of the corrections described in paras. 1.6.2 and 1.6.3, and shown partly in Fig. 2, are as follows:

E = displacement as indicated on the displacement meter (cm)
E_i = initial value indicated on the displacement meter (cm)
E_u = last displacement as indicated on the displacement meter (cm)
E^* = calculation constant of the displacement meter (cm)
F = 50.26 cm^2 = total cross-sectional area perpendicular to juice flow (cm^2)
F_L = 26 cm^2 = surface of the juice in the receiver used in the pressing operation (cm^2)
h_r = water vapour tension at the temperature of the measuring room (mmHg)
h_{25} = water vapour tension at 25 °C (mmHg)
p_0 = corrected total pressure (kp/cm^2 gauge pressure)
q_s = corrected juice yield calculated from the values weighed on the balance (pond)

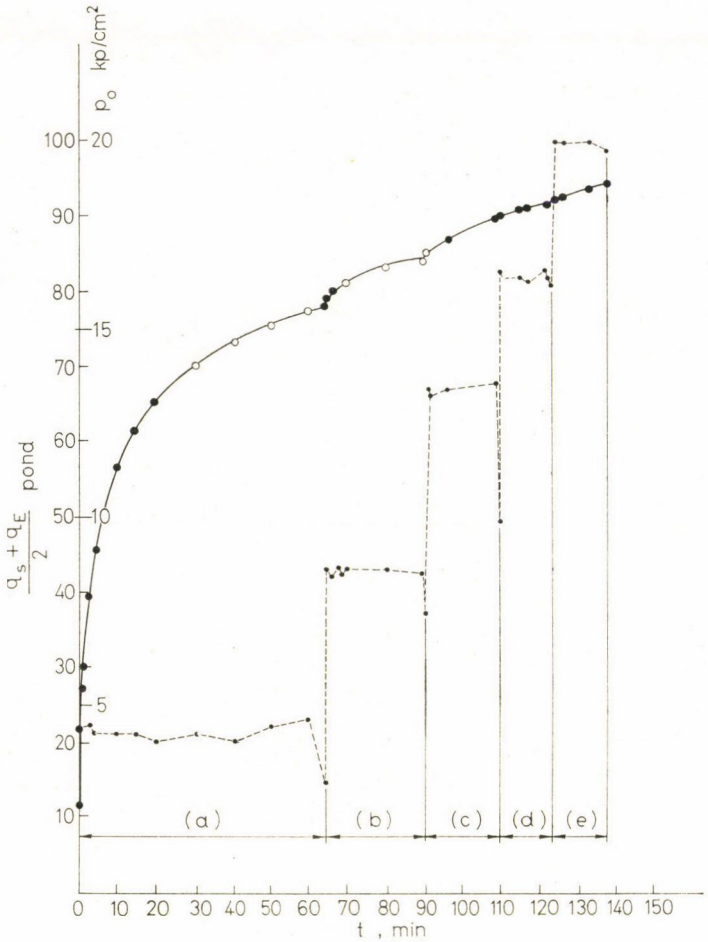

Figure 3. Corrected juice yield and pressure values vs. pressing time. First measurement on January 23. Juice yields are marked by solid and open circles, pressure by a dotted line. The values pertaining to the open circles were used in the calculation of equilibrium juice yield

q_E = corrected juice yield calculated from the data of the displacement meter (pond)

q_{Eu} = the value of q_E at time t_u (pond)

q_i = weight of pulp charged into the apparatus (pond)

q_m = weight of juice as indicated on the balance (pond)

q_p = weight of juice lost through evaporation (pond)

q_{pu} = weight of juice lost through evaporation at time t_u (pond)

q_t = weight of pressing cake after pressing (pond)

q_{mu} = the last weight indicated on the balance at time t_u on determining the correlation between juice yield and time (pond)

Δq_m = juice absorption by the filter cloth and by the supporting elements (pond)
t = pressing time (minute)
t_u = last pressing time on determining the juice yield vs. time correlation (minute)
v_p = evaporation rate (pond per minute)
γ_f = specific gravity of the expressed juice (pond per cm^3)
φ = relative humidity in the room in which the measurements are performed.

In agreement with Fig. 2 and the symbols used the following simplifications were applied to calculate the corrections of juice yield: When pressure is applied to the pulp at $t = 0$ the piston occupies position 1, after which the piston expels in practically no time the air from the pulp (position 2). The next theoretically separate phase would be the absorption of the juice by the filter cloth and the supporting elements (position 3). This operation takes place, in fact, simultaneously with the displacement of air and therefore the time is still $t = 0$. After this the juice begins to appear on the balance (position 4), and the conditions at the end of pressing are shown by position 5. Evaporation from the receiver and from the filter surface is proportional to pressing time.

1.6.2. Calculation of the juice yield corrections. The data obtained from the displacement meter were transformed into values of juice yield (weight) by accounting for the postulations of para. 1.6.1 with the help of the following equations:

$$q_E = (E - E^*)F\gamma_f \tag{1}$$

and

$$E^* = E_u - \frac{q_i - q_t}{F\gamma_f} \tag{2}$$

The calculation constant E^* stands for the value indicated on the displacement meter at the position of the piston when the air has already been expelled but no juice had yet flown from the pulp (see position 2 in Fig. 2).

The corrected juice yields were obtained from the juice yield values measured on the balance with the help of the following equations:

$$q_S = q_m + \Delta q_m + q_p \tag{3}$$

$$\Delta q_m = q_i - q_t - (q_{mu} + q_{pu}) \tag{4}$$

$$q_p = v_p \cdot t \tag{5}$$

$$q_{pu} = v_p \cdot t_u \tag{6}$$

The corrections applied in the above manner lead to the following results:

1. The corrected juice yield pertaining to time t_u is equal to the value of $q_i - q_t$.

2. The corrected juice yield pertaining to time t_u is equal to the juice yield calculated by means of the displacement meter, i.e.

$$q_{Eu} = q_i - q_t \tag{7}$$

1.6.3. Accounting for evaporation. The evaporation rate v_p in para. 1.6.2 was determined by calculation using the following formula:

$$v_p = 3.66 \cdot 10^{-5} \left[\frac{F + F_L}{2} (h_{25} - \varphi h_r) + \frac{F_L}{2} (1 - \varphi) h_r \right] =$$

$$= (33.15 + 0.475\, h_r - 1.875\, \varphi h_r) \cdot 10^{-3} \frac{\text{pond}}{\text{min}} \tag{8}$$

The interpretation of the symbols and the values were given in para. 1.6.1. In this formula evaporation both on the filter cloth and from the receiver have been accounted for. Quite briefly, the formula was obtained as follows: the dimensional constant is the evaporation coefficient at zero air velocity for horizontal water surfaces in the LURIE—MIHAILOFF correlation (PÉCH, 1963).

Re-arrangement of the term in the angular brackets shows that at the filter surface a temperature of 25 °C was assumed (temperature of the thermostat) and here half of the mentioned evaporation coefficient was used in the calculation. This fact was supported by preliminary experiments details of which are not given here. Since the vapour leaves the filter surface in a downward direction and is at the same time surrounded by the cylindrical jacket it is understandable that compared to the LURIE—MIHAILOFF value the evaporation coefficient will be lower. In the receiver the temperature of the juice must be between 25 °C and room temperature, further the vapours leave the surface of the liquid in an upward direction. For this reason here the mean of the difference between water vapour tension at the surface of the juice (25 °C and room temperature) and in the measuring room were taken into account and an evaporation coefficient corresponding to the dimensional constant was used.

1.6.4. Accounting for juice leakage. The value of leakage is the weight of juice on the balance leaking from the pulp prior to the application of pressure. In the evaluation of the experiments this quantity is accounted for as if it had appeared immediately after the application of pressure, provided that leaking is but slight. In this case it can, namely, be assumed that the structure of the material remains unchanged and leaking takes place from the parts in direct

contact with the filter cloth. In the initial sections of the juice yield vs. time curves juice yield increases at a very high rate. If leaking could be prevented then this amount of juice would have appeared on the balance in the first few seconds of pressing.

1.6.5. Correction of the measured value of pressure. The measured value of pressure is reduced by the pressure necessary to overcome the friction of the piston. The friction of the piston was determined in separate experiments and was found to have a value of 0.368 kp/cm^2.

1.7. Method of measurements

From apples stored and processed according to paras. 1.2 and 1.3 125 ponds were weighed and filled in the pressing chamber in the head as shown in Fig. 1. The few tenth of a pond losses occurring during weighing were accounted for. Care was taken that the time passing between grinding and pressing should always be the same.

The pulp in the pressing head was covered with filter cloth and turned in the direction of the balance. 0.08 pond of tannine and 0.08 pond of phosphorus tungstic acid were placed in the receiver. The desired pressure adjusted, and the chronometer set into motion, the instruments were photographed. In the instant of exposition the valve closing the connecting pipeline between the pressing head and the pressure reducing valve was opened whereby the pulp was put under pressure. During measurement the pressure was adjusted in accordance with the requirements. The instruments were photographed at determined intervals.

After the completion of pressing the pressure was released, the cake carefully collected and its weight (q_t) determined. The juice obtained by this operation was added to the juice obtained in previous measurements and stored in a closed jar at +10 °C. The first portion was set apart on January 23, the last on January 28.

The soluble solids, viscosity, fibre content and specific gravity were determined (according to paras 1.4 and 1.5) on January 29. After the addition of the last portion the tannine and phosphorus tungstic acid contents were made up to 0.1% each.

The juice yield was calculated according to para. 1.6, from the data of weight and displacement measurements (q_s and q_E) and average $\frac{q_s + q_E}{2}$ was formed. Because of the applied method of photographing, the measurement of time can be considered practically free of error, while it is impossible to ascertain which of the two methods for the determination of juice yield is more accurate, hence the average is given as the most probable approximation of the actual value.

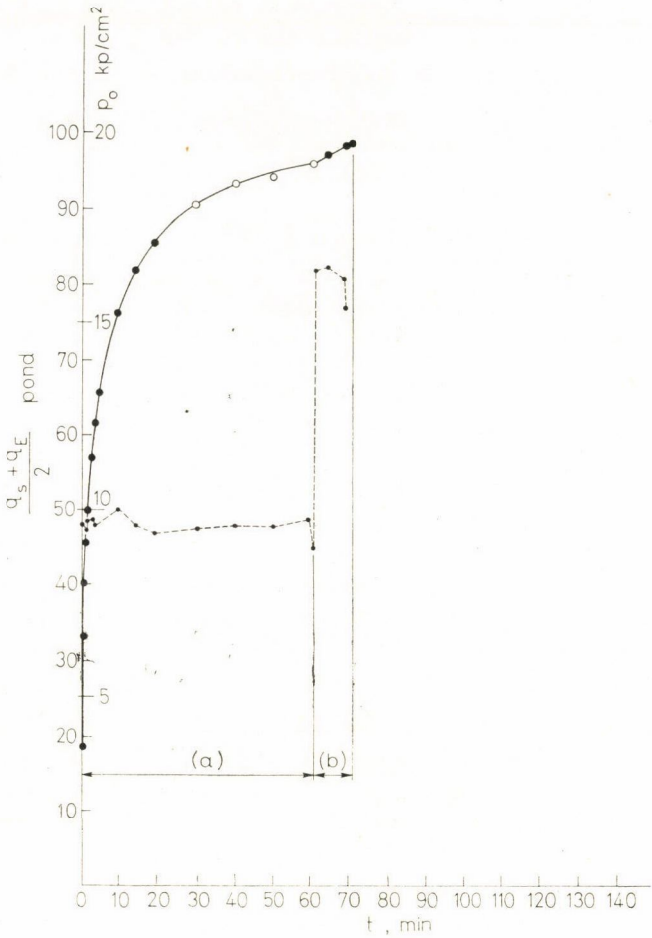

Figure 4. Corrected juice yield and pressure values vs. pressing time. Second measurement on January 23. Juice yields are marked by solid and open circles, pressure by the dotted line. The values pertaining to the open circles were used in the calculation of equilibrium juice yield

2. Results

The date and main data of the measurements are given in Table 1. Table 2 contains the data of viscosity, soluble solids content, fibre content and specific gravity as well as the air content of the pulp.

The average of the corrected juice yields calculated according to para. 1.6, $\left(\frac{q_s+q_E}{2}\right)$, as well as the corrected values of pressure (p_0) are plotted in Figs 3, 4, 5, 6, 7 and 8.

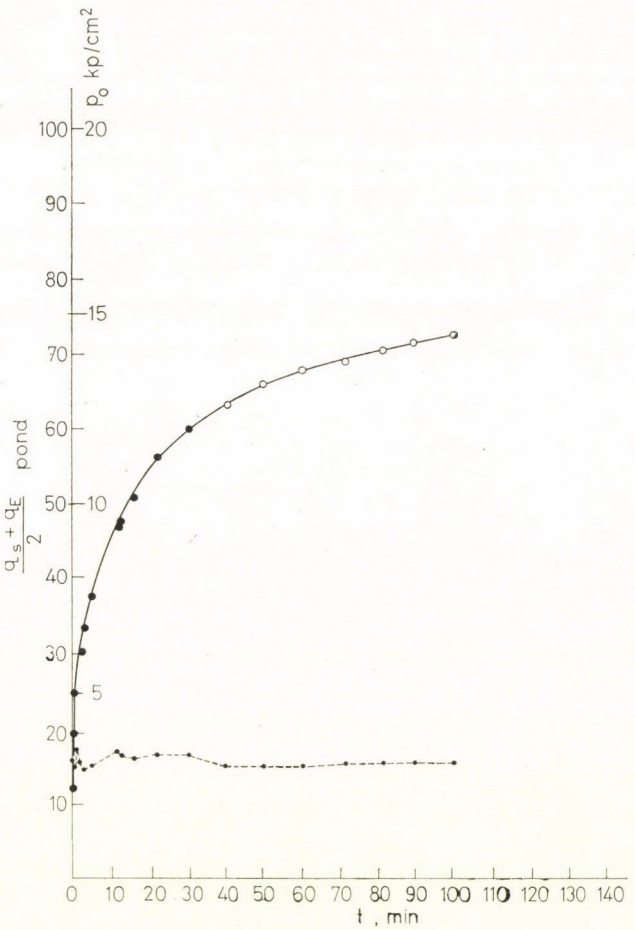

Figure 5. Corrected juice yields and pressure values vs. pressing time. First measurement on January 26. Juice yields are marked by solid and open circles, pressure by the dotted line. The values pertaining to the open circles were used in the calculation of equilibrium juice yield

The juice yield values employed in the calculation of equilibrium juice yields (q_0) are marked separately in the Figures. In some juice yield curves different sections (a), (b), (c) . . . were distinguished. The pressure has been altered at the beginning or the end of these sections. The juice yield, time and pressure values used in the determination of the correlation between equilibrium juice yield and pressure are shown separately in Table 3. The temperature and relative humidity of the room in which the pressing experiments were carried out are given in Table 4.

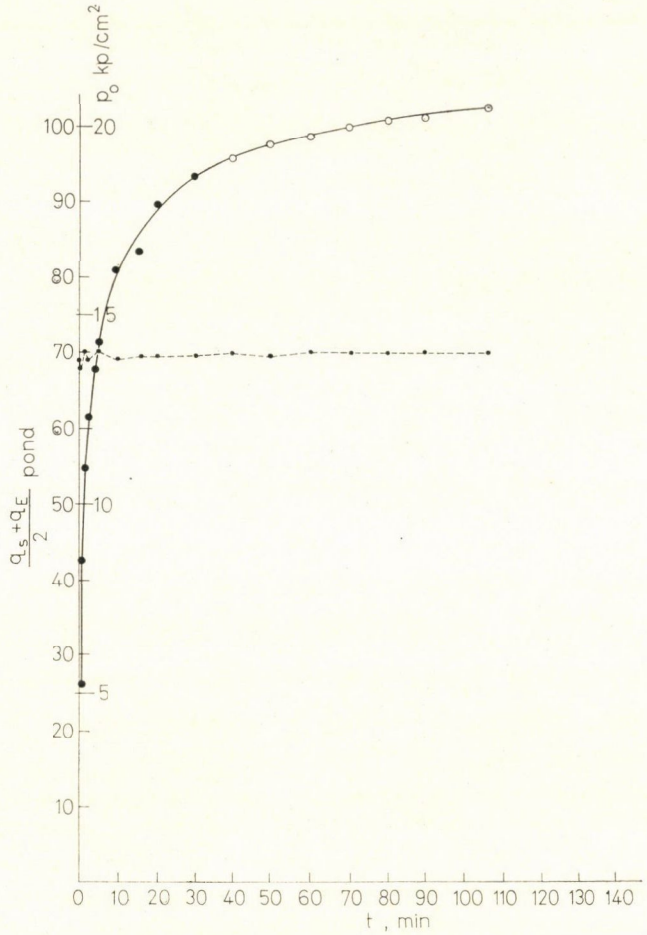

Figure 6. Corrected juice yield and pressure values vs. pressing time. Second measurement on January 26. Juice yields are marked by solid and open circles, pressure by the dotted line. The values pertaining to the open circles were used in the calculation of equilibrium juice yield

3. Conclusions

3.1. Evaluation of corrections

3.1.1. The calculation constant of the displacement meter. The calculation constant of the displacement meter (E^*) is given in Table 1. The average value is 3.4307 cm, the greatest deviation from average is 0.0607 cm, empirical standard deviation $s = \pm 0.0283$ cm, or $\pm 0.82\%$ of the average value. It is important to know that when the piston was in the initial position, the displacement meter showed in all the measurements listed in Table 1 a value of

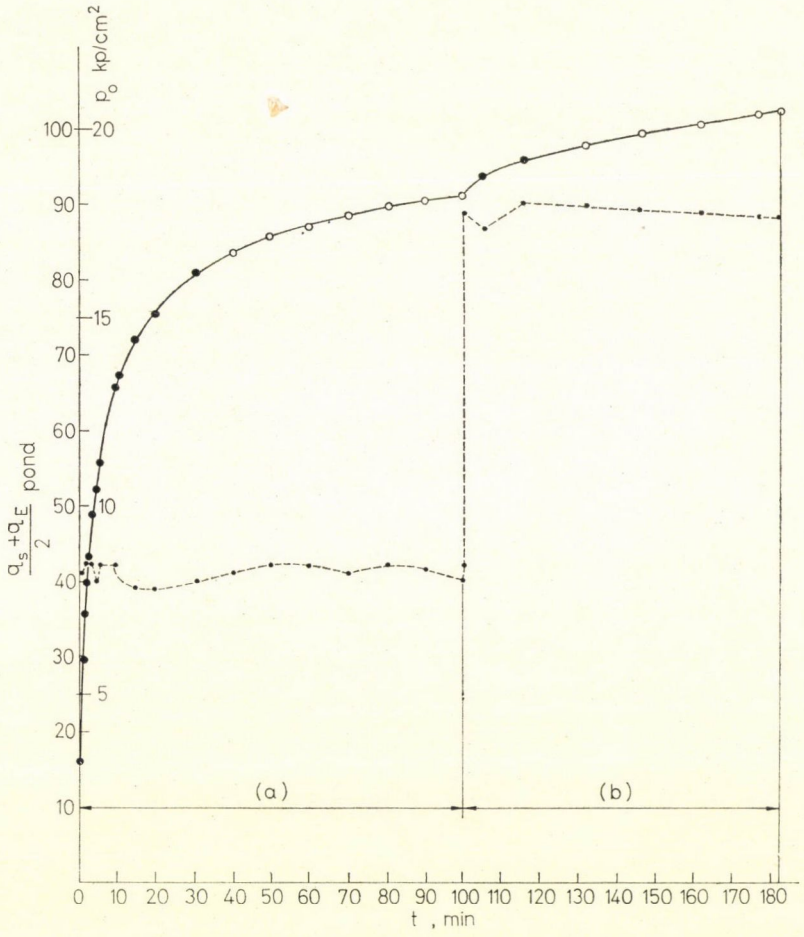

Figure 7. Corrected juice yield and pressure values vs. pressing time. First measurement on January 27. Juice yields are marked by solid and open circles, pressure by the dotted line. The values pertaining to the open circles were used in the calculation of equilibrium juice yield

$E_i = 3.00$ cm, thus the displacement necessary to remove the air content of the pulp was on the average $3.4307 - 3.00 = 0.4307$ cm, and the air content of the pulp was 21.65 cm³ which corresponds to an air volume of 0.1735 cm³ per pond of pulp weight.

3.1.2. Correction for evaporation. The evaporation rate was calculated from Eq. 8 using the data in Table 4. Since there was no significant difference in the evaporation rates of various measurements, for the sake of simplicity (since we are anyhow dealing with values obtained by calculation) the mean value of the various evaporation rates, $v_p = 0.0209$ pond/min was taken into consideration.

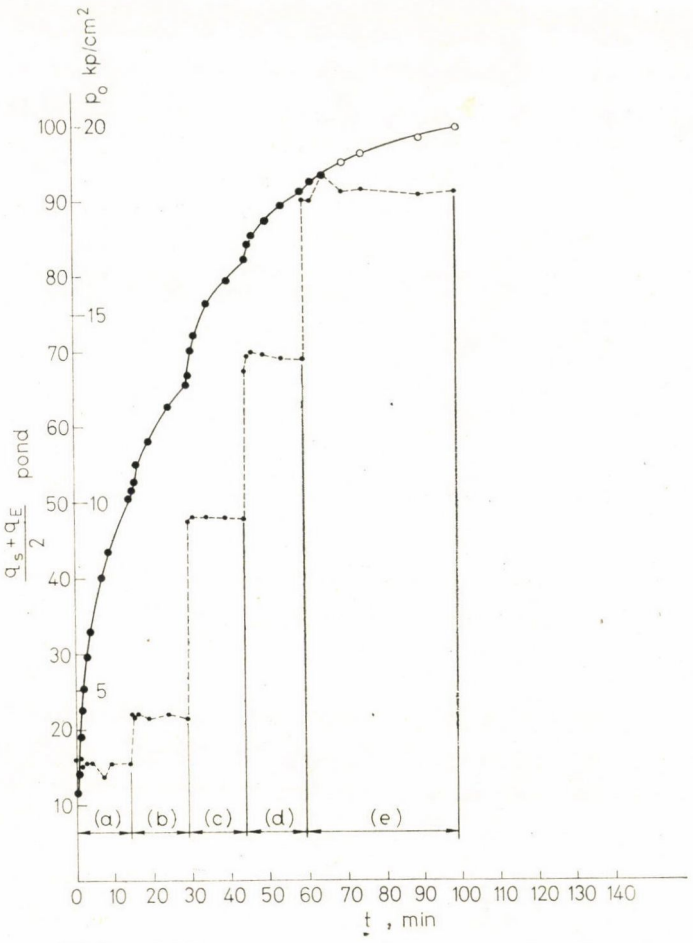

Figure 8. Corrected juice yield and pressure values vs. pressing time. Second measurement on January 28. Juice yields are marked by solid and open circles, pressure by the dotted line. The values pertaining to the open circles were used in the calculation of equilibrium juice yield

3.1.3. Juice absorption by the filter cloth and by the supporting elements. This value was calculated from Eq. 4. Values q_i, q_t and q_{pu}, needed for the calculation, are shown in Table 1. The q_{pu} values were obtained by multiplying the evaporation rate according to para. 3.1.2 with the last pressing period (t_u). From the measurements the average of

$$\overline{\Delta q_m} = 5.67 \text{ pond}$$

was obtained for the juice absorption of the filter cloth and of the supporting elements, with $s = \pm 1.495$ pond as the empirical corrected standard deviation.

Table 1

Summary of measurements
Material: apple pulp. Temperature of pressing: 25 °C

1.	2.	3.	4.	5.	6.	7.	8.
January 23, 1st measurement	124.9	30	3.461	33.5	5.34	137	2.86
January 23, 2nd measurement	124.8	25.9	3.433	28	7.42	70.5	1.48
January 26, 1st measurement	124.8	52	3.370	33	5.21	100	2.09
January 26, 2nd measurement	124.6	23	3.437	32	5.78	106	2.22
January 27, 1st measurement	124.8	22.7	3.444	34	3.3	182	3.80
January 28, 2nd measurement	124.9	25.2	3.437	33	7.13	99	2.07
Average	124.8	—	3.4307	32.25	5.67		

1. Date and symbol of measurement
2. Weight of pulp charged in the press, q_1 pond
3. Weight of cake, q_t pond
4. Calculation constant of displacement meter, E^* cm
5. Time interval between grinding and pressing, minutes
6. Juice absorption of filter cloth and supporting elements, Δq_m pond
7. Last pressing time, t_u minutes
8. Evaporation loss for t_u pressing time, q_{pu} pond

Table 2

Viscosity, soluble solids content, fibre content, specific gravity of the pressed apple juice and the air content of the pulp

Viscosity, cP	3.43
Soluble solids content, refractive index, %	12.5
Fibre content, %	5.76
Specific gravity, pond/cm³	1.05
Volume of air per weight of air-free pulp, cm³/100 pond	17.34

3.1.4. Discrepancy between the values calculated from weight and displacement measurements, and an example for the calculation of corrections and of the juice yield displacement measurement. When derived from formula (1) and (3) no significant difference was found between juice yields q_E and q_S. Table 5 shows the absolute value of the maximum deviations of individual measurements $(q_E - q_S)$. Deviation from the mean value $\frac{q_S + q_E}{2}$ is obviously the half of the latter. It appears from Table 5 that even the maximum deviation (2nd measurement on January 26) did not amount to more than 6.35 % of the pulp charge. It appears further from the Table that maximum deviations occur in the initial period of the measurement. With the exception of the measurement on January 28 (19 minutes) these time values vary between 0.07 and 2 minutes.

Table 3

Data used in establishing the correlation between equilibrium juice yield and pressure

Date and symbol of measurement	Pressing time t, min	Mean value of corrected juice yields, $\frac{q_S+q_E}{2}$, pond	Corrected pressure P_0, kp/cm²
1	2	3	4
January 23,	30	70.420	4.23
1st measurement	40	73.440	4.03
section (a)	50	75.895	4.43
	60	77.695	4.63
section (b)	69.5	81.445	8.63
	79.5	83.600	8.63
	89.5	84.755	8.53
	90	85.560	7.43
January 23,	30	90.775	9.53
2nd measurement	40	93.530	9.63
section (a)	50	94.485	9.63
	60	96.440	9.83
January 26,	40	63.425	3.03
1st measurement	50	66.130	3.03
	60	67.780	3.03
	71	69.395	3.13
	81	70.800	3.13
	90	71.645	3.13
	100	72.800	3.13
January 26,	40	95.810	14.03
2nd measurement	50	97.565	13.93
	60	98.665	14.03
	69.9	99.820	14.03
	80	100.675	14.03
	90	101.330	14.03
	106	102.500	14.03
January 27,	40	83.420	8.23
1st measurement	56	85.625	8.43
section (a)	60	86.975	8.43
	70	88.330	8.23
	80	89.735	8.43
	90	90.340	8.33
	100.5	91.250	8.43
section (b)	132	97.73	17.93
	147	99.185	17.83
	162	100.345	17.73
	177	101.550	17.63
	182	102.100	17.63
January 28,	69	95.035	18.23
2nd measurement	74	96.190	18.33
section (e)	89	98.295	18.13
	99	99.700	18.23

The value of $\overline{\Delta q_m}$ is 4.54% of the pulp charged into the pressing head (q_i) and is thus by no means negligible.

Table 4

Temperature and relative humidity of the laboratory during measurements

Date and symbol of measurements	Temperature °C	Relative humidity %
January 23, 1st measurement	16.5—18	68—68.5
January 23, 2nd measurement	18	69
January 26, 1st measurement	14—14.8	69—71
January 26, 2nd measurement	15.7	71
January 27, 1st measurement	17.8	71.5
January 28, 2nd measurement	18	66.5

The deviations $(q_E - q_S)$ are plotted in Figs 9 and 10 as the function of time. We can see that at the initial and end points of the curves $(t = 0, t = t_u)$ the deviations are equal to zero. This follows from the principle of the applied correction method.

Next an example will be given for the calculation of the juice yields and corrections. On January 23, this being the first measurement, the following values were measured at $t = 90$ minutes pressing time:
The weight of juice yield as indicated on the balance: $q_m = 78.5$ pond
Value indicated on the displacement meter: $E = 5.08$ cm
Evaporation loss at $t = 90$ minutes: $q_p = 0.0209 \times 90 = 1.88$ pond
Quantity of the pulp charge: $q_l = 124.9$ pond
Weight of pressing cake: $q_t = 30.0$ pond
Last pressing time: $t_u = 137$ minutes
Juice yield obtained at the last pressing time: $q_{mu} = 86.7$ pond

Table 5

Absolute value of the greatest difference between corrected juice yield calculated from the weight and juice yield calculated from displacement measurement in the various experiments

Date and symbol of experiment	maximum$\lvert q_E - q_S \rvert$ pond	$100 \cdot \frac{\max.\lvert q_E - q_S \rvert}{q_l}$ %	Pressing time, min
January 23, 1st measurement	3.63	2.90	2
January 23, 2nd measurement	5.98	4.79	0.07
January 26, 1st measurement	5.55	4.45	2
January 26, 2nd measurement	7.92	6.35	0.07
January 27, 1st measurement	5.68	4.55	1
January 28, 2nd measurement	3.43	2.75	19

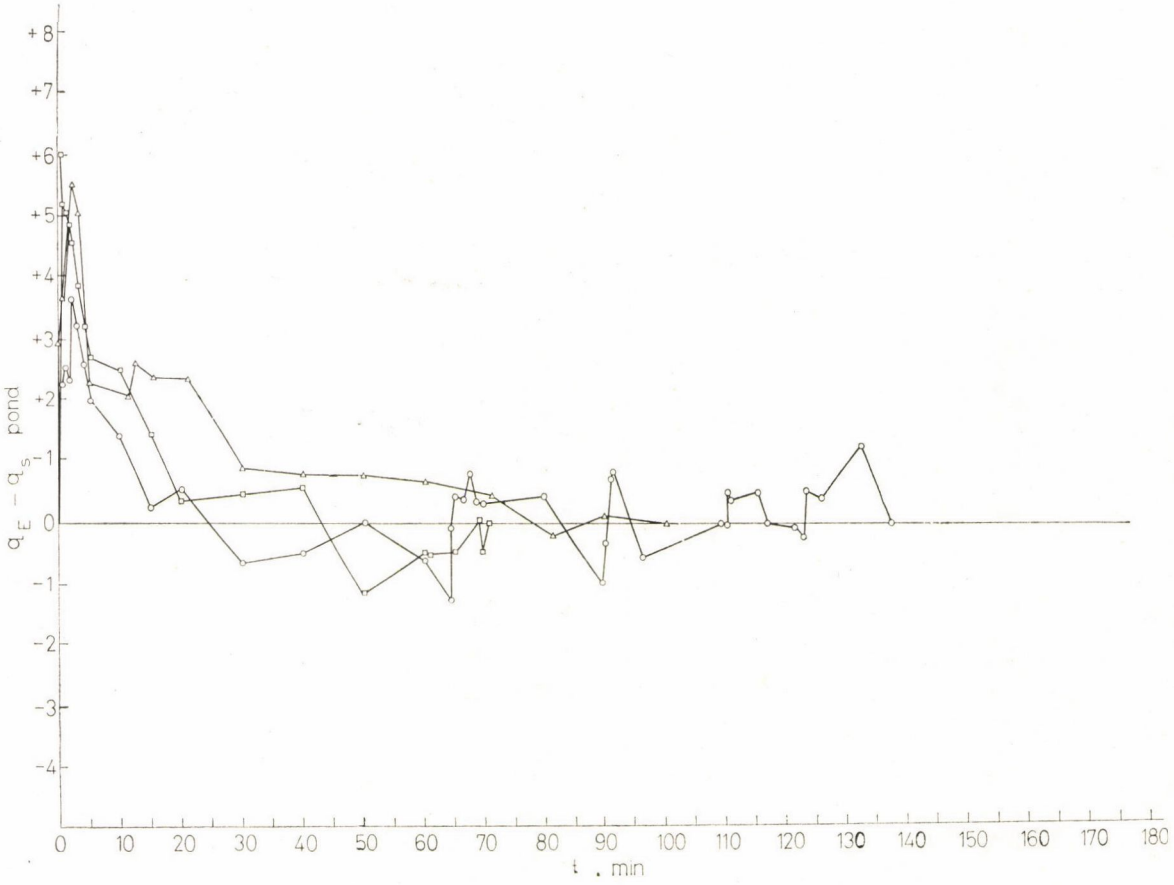

Figure 9. Difference between juice yields calculated from the displacement-meter values and those calculated from weights vs. pressing time: open circle (○): 1st measurement, January 23; open square (□): 2nd measurement, January 23; open triangle (△): 1st measurement, January 26

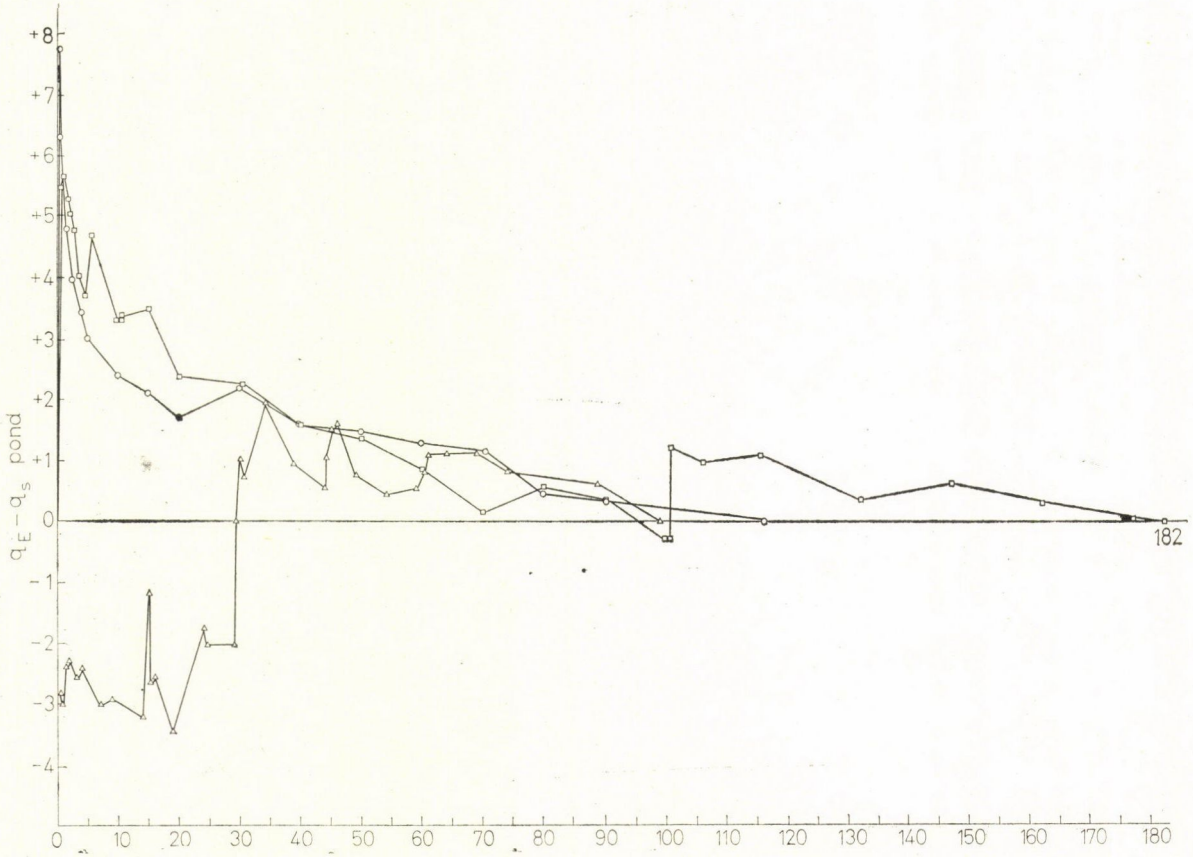

Figure 10. Difference between juice yields calculated from the displacement meter values and those calculated from weights vs. pressing time: open circle (○): 2nd measurement, January 26; open square (□): 1st measurement, January 27; open triangle (△): 2nd measurement, January 28

Displacement measured at the last pressing time: $E_u = 5.26$ cm
Evaporation loss at $t_u = 137$ minutes: $q_{pu} = 0.0209 \times 137 = 2.86$ pond

Further data are given in Table 2. The calculation constant of the displacement meter is from Eq. (2):

$$E^* = 5.26 - \frac{124.9 - 30}{50.26 \times 1.05} = 5.26 - 1.799 = 3.461 \text{ cm}$$

The juice yield calculated from the displacement value through Eq. (1):

$$q_E = (5.08 - 3.461) \times 50.26 \times 1.05 = 85.4 \text{ pond}$$

From Eq. (4) the juice absorption of the filter cloth and of the supporting elements is:

$$\Delta q_m = 124.9 - 30 - (86.7 + 2.86) = 5.34 \text{ pond}$$

From Eq. (3) the corrected juice yield obtained by weight measurement is:

$$q_s = 78.5 + 5.34 + 1.88 = 85.72 \text{ pond}$$

The mean value of juice yields calculated by the two different methods (hereafter corrected juice yield) is:

$$\frac{q_s + q_E}{2} = \frac{85.72 + 85.4}{2} = 85.56 \text{ pond}$$

The difference between the juice yields calculated by the two different methods is:

$$q_s - q_E = 0.32 \text{ pond}$$

3.2. Determination of the correlation between equilibrium juice yield and pressure

Experience has shown that a very prolonged pressing period would be needed to reach the equilibrium juice yield (q_0) pertaining to a given pressure.

It can be assumed that an infinitely long time is required for reaching equilibrium juice yield (KÖRMENDY, 1968). It is, however, impossible to use too long pressing periods, since these would certainly cause some changes in the material to be pressed, due partly to the activity of microbes and partly to that of enzymes. It is therefore necessary to draw conclusions with respect to equilibrium juice yield from the data obtained during a pressing period not longer than maximum one or two hours. The calculation method to be de-

scribed below is based on the assumption that at a given constant pressure the equation describing juice yield as a function of pressing time will be as follows:

$$q = q_0 (1 - ae^{-bt}) \tag{9}$$

Now the next task is to determine the values of q_0, a and b in such a way that the sum of the squares of the differences between the corrected juice yields calculated from the measurements and the juice yields calculated from Eq. (9) shall be the minimum, that is the minimum of the sum

$$M = \sum_{j=1}^{N} (q - q_j)^2 \tag{10}$$

has to be formed. The following steps were used:

1. Taking the value of b constant
2. Equations

$$\frac{\partial M}{\partial q_0} = \frac{\partial}{\partial q_0} \sum_{j=1}^{N} (q - q_j)^2 = 0 \tag{11}$$

and

$$\frac{\partial M}{\partial a} = \frac{\partial}{\delta a} \sum_{j=1}^{N} (q - q_j)^2 = 0 \tag{12}$$

were formulated.

3. The solution of Eqs (11) and (12) leads to

$$a = \frac{\sum_1^N q_j \sum_1^N f_j - N \sum_1^N q_j f_j}{\sum_1^N q_j \sum_1^N f_j^2 - \sum_1^N f_j \sum_1^N q_j f_j} \tag{13}$$

and

$$q_0 = \frac{\sum_1^N q_j f_j}{\sum_1^N f_j - a \sum_1^N f_j^2} \tag{14}$$

where the symbol: $f_j = e^{-bt_j}$ was used.

4. By substituting the values a and q_0 into Eqs (9) and (10) the value of M is obtained.

Carrying out this calculation for various values of b and plotting the value of M as a function of b, a curve is obtained, on which the minimum value of M can be found.

Fig. 11 shows the a, q_0 and M values calculated from the data of the first measurement on January 23 [Table 3, section (a)] and the values of a, b and q_0 pertaining to $M_{\min}$ are marked.

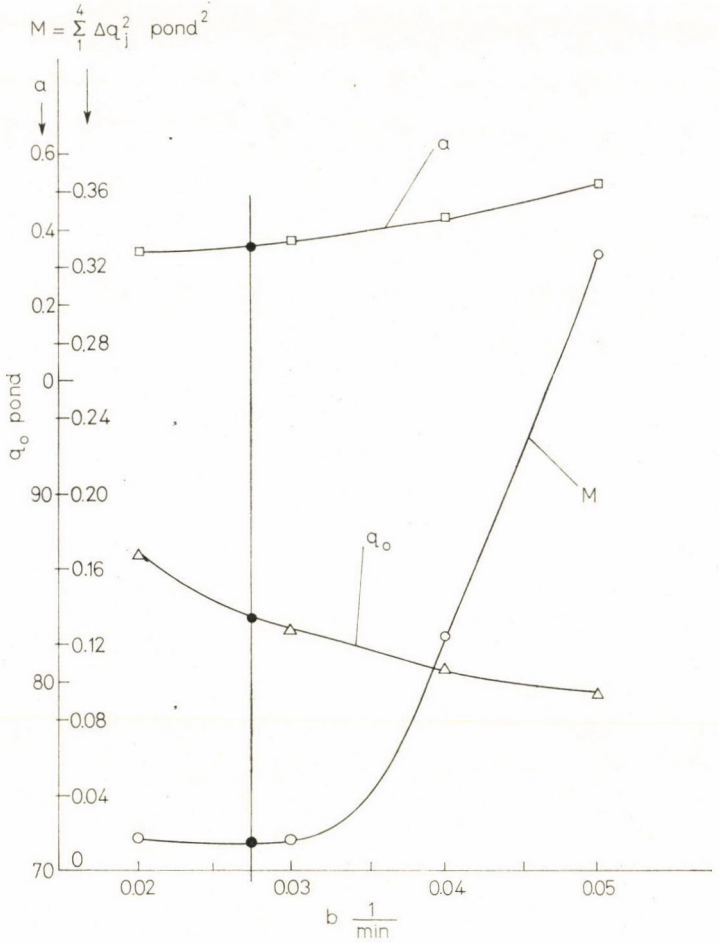

Figure 11. Equilibrium juice yield (q_0), a and the sum of squares of the deviations (M as functions of b. Calculated from the points marked by open circles in section (a) o the 1st measurement on January 23. The open circles mark the values of M, the open triangles those of q_0 and the open squares those of a. At the minimum of M $b = 0.0275$ min^{-1} and the pertaining q_0, a and M values are marked by solid circles

5. The q_0 value pertaining to M_{min} is considered as the equilibrium juice yield.

Performance of such a great number of calculations manually would be too lengthy and inaccurate, therefore a Hewlett—Packard, type 9100B electronic table computer was programmed for the purpose.

Within each section of measuring the pressure was kept as far as possible at a constant value. These sections are marked on Figs 3 to 8, and the symbols of the sections are also shown in Table 3. Including the measurement performed on January 26, a total of 8 such sections were used involving 4 to 7 measure-

ment points. The values of q_0, a and b calculated from the various sections, as well as the average of the corrected pressures pertaining to the used points of measuring in the sections are given in Table 6. The points are at the end of the sections, and are marked in Figs 3 to 8 and in Fig. 12 by open circles.

The standard deviation, $s = \pm \sqrt{\frac{M}{N-2}}$ is also given for each section. The question is now how the points of measurement on which calculation is based have been selected, and what were the considered aspects in the selection of the b values, including the one pertaining to the minimum.

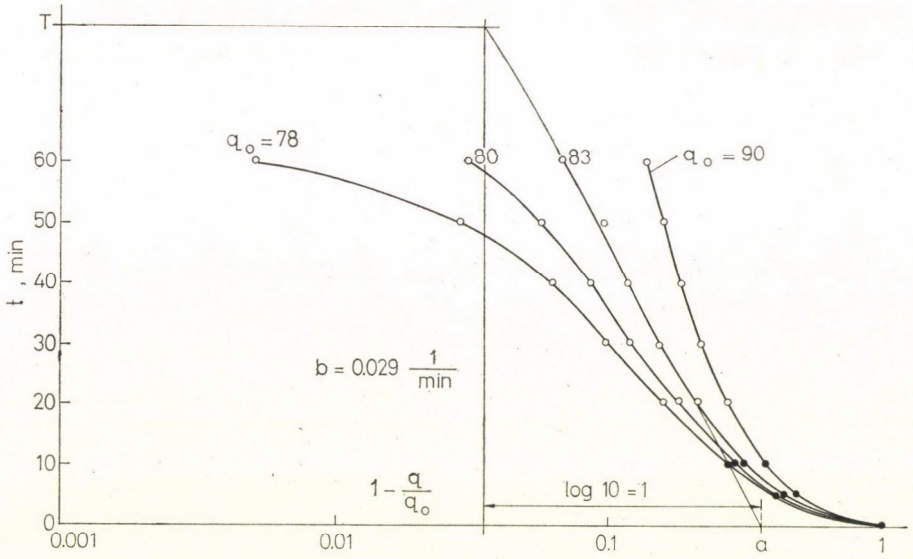

Figure 12. Logarithm $\left(1 - \frac{q}{q_0}\right)$ vs. pressing time for various q_0 values. The open circles mark the points used for the determination of equilibrium juice yield

Table 6

Equilibrium juice yields, average of corrected pressure in the sections, standard deviations and the characteristic constants of the measurement sections according to Eq. (9)

Date and symbol of measurement		q_0 pond	p_0 gauge pressure kp/cm²	s pond	a	b 1/min
January 23, 1st measurement	(a)	83.4	4.330	±0.0895	0.352	0.0275
	(b)	89.62	8.305	±0.37	0.733	0.03
January 23, 2nd measurement	(a)	110.25	9.655	-±0.427	0.220	0.029
January 26, 1st measurement		77.0	3.087	±0.1611	0.374	0.019
January 26, 2nd measurement		105.1	14.015	±0.1105	0.186	0.019
January 27, 1st measurement	(a)	94.5	8.356	±0.1455	0.26	0.020
	(b)	106.0	17.750	±0.21	0.655	0.01575
January 28, 2nd measurement	(e)	105.8	18.230	±0.1415	0.37	0.019

As already mentioned, Eq. (9) can be used in the case of constant pressure and only if the pressing period is sufficiently long. To decide the shortest, but still satisfactorily long pressing period, a graphic method was used, as illustrated by Fig. 12. This Figure shows the points of measurement in section (a) of the first measurement on January 23. Pressing periods were plotted on the normal axis (ordinate) of a semi-logarithmic graph paper, while the values $1 - q_j/q_0$ were plotted on the logarithmic scale abscissa. Here q_0 was chosen arbitrarily but preferably slightly above the highest measured q_j value (in our case 77.7 pond). Each of the group of curves shown in the Figure was obtained in this way, e.g. in case of $q_0 = 78$ pond, the first curve on the left. By choosing another q_0 a new curve was obtained, etc. All curves have been marked with the q_0 parameter used for their calculation.

Since the values of q and t fulfilling the conditions of Eq. (9) are, if plotted in this manner, on a straight line, we have to find now from the group of curves that one whose final section (at longer pressing periods) is the first one to straighten out when proceeding from left to right. The q_0 value pertaining to this curve will presumably closely approximate the value calculated with the help of Eq. (14). Prolongation of the straight section intersects the abscissa giving a value for a which is expected to lie close to the value calculated from Eq. (13). The value of b is determined from the equation

$$b = 2.3026 \frac{1}{T}$$

For the calculations we have chosen values greater and smaller than this. Measurement points to be substituted into Eqs 10 to 14 shall be those which, if regressing from the longest pressing time will visibly lie on the straight line or scatter round it. If there is some doubt whether a further point shall also be involved the calculation with Eqs 10 to 14 is repeated by accounting for this new point of measurement too. Should the standard deviation decrease or remain unchanged, this new measuring point might be included in the calculation.

Referring to section (a) of the first measurement performed on January 23 the following values were obtained by means of the graphic process and by calculation:

	graphic procedure	calculation
q_0	83	83.4
a	0.36	0.352
b	0.029	0.0275

As seen the graphic method, too, gives a satisfactory approximation.

By dividing the q_0 values from the data in Table 6 with the average charge weight of $\bar{q}_i = 124.8$ pond the percentage equilibrium juice yields are obtained which have been plotted vs. pressure in Fig. 13. A correlation

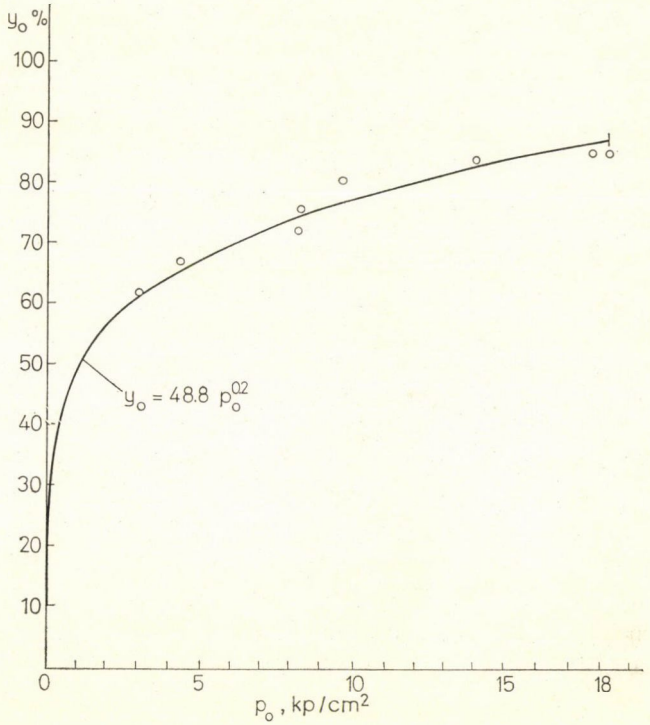

Figure 13. Percentage juice yield (y_0) vs. pressure (p_0). The open circles mark the points calculated from the measurements, the curve represents correlation $y_0 = 48.8\ p_0^{0.2}$

$$y_0 = A\ p_0^B$$

was assumed to exist between the percentage juice yield and the pressure and constants A and B were determined by the method of least squares leading to

$$y_0 = 48.8\ p_0^{0.2} \tag{15}$$

(the value of y_0 is given in per cent, that of p_0 in kp/cm²).

This correlation has been plotted in Fig. 13, too, the standard deviation is:

$$s = \pm \frac{\sum_1^N (y_{0j} - y_0)^2}{N - 2} = \pm 2.4\% \text{ of the juice yield.}$$

Equation (15) is valid only for $0 < p_0 < 18.23$, that is extrapolation beyond maximum pressure is forbidden.

This approximating relationship has been chosen because it is easy to handle, it goes through the points $y_0 = 0$ and $p_0 = 0$ and because in this point, in good agreement with experience, the differential quotient of per-

enhtage equilibrium juice yield per pressure is infinite. Experience has shown ctat at quite small pressures the equilibrium juice yield increased very rapidly. Use of the above correlation involves the drawback of not expressing the condition that at increasing pressure y_0 approaches a limit value. Of course, approximations by other functions might also be possible.

3.3. Symbols

The symbols not included in para. 1.6.1 are given below:

a = constant in Eq. (9) (dimensionless)
A = constant in Eq. (15) (per cent/(kp/cm^2)$^{0.2}$)
b = constant in Eq. (9) (1/min)
f_j = e^{-bt_j} (dimensionless)
M = sum of squares of juice yield deviations according to Eq. (10) (pond^2)
N = number of points of measurement
q = corrected juice yield in general (pond)
$\bar{q}_i$ = 124.8 pond = average of charged weights
q_j = value of corrected juice yield calculated from a single measured data (pond)
q_0 = equilibrium juice yield (pond)
s = standard deviation (empirical corrected deviation, dimension the same as that of its variant)
t_j = a single measured data of pressing time (min)
T = time constant according to Fig. 12 (min)

$y_0 = 100 \dfrac{q_0}{\bar{q}_i}$, percentage equilibrium juice yield (%)

$y_{0j} = \dfrac{q_{0j}}{\bar{q}_i} 100$ = percentage equilibrium juice yield calculated from a single measured data (%)

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A DERIVATOGRAPHIC STUDY OF THE THERMAL DECOMPOSITION OF GLUCOSE

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The thermal decomposition of glucose was studied in the Derivatograph of Paulik, Paulik and Erdey ensuring reproducible heat treatment. In the Derivatograph changes taking place in the course of heat treatment can be read off the simultaneously recorded curves of sample weight, the rate of weight change, temperature of the sample and change in enthalpy. The information obtained from the derivatograms, supplemented with the results of the analyses of volatile decomposition products and of the residue, have led to the following results:

1. In a glucose sample mixed with twice its amount of alumina and heated at a rate of 6 °C per minute the formation of caramel begins at 165 °C with the melting of glucose and at 240 °C the entire quantity of glucose is converted into caramel.

2. In the temperature range from 240° to 300 °C the caramel colouring substance begins to pyrolyze, hence in the study of caramel formation this temperature range should be avoided as much as possible.

3. Derivatograms of some intermediate products of caramel formation and of wheat starch, plotted under the same conditions as the derivatogram of glucose, suggest, in addition to the above findings, the formation of "reversion products" (polysaccharide) beside the colouring matter and the presence of 5-hydroxymethyl-furfural among the products volatilizing at 200 °C.

In the study of caramelization, i.e. of the thermal decomposition of sugar, the reproducibility of heat treatment presents a difficult problem (TELEGDY KOVÁTS, 1955) where the Derivatograph is of considerable help. The Derivatograph is an automatically operating derivating thermobalance which, beyond providing for the reproducible heat treatment of the sample, is also capable of recording the change of weight and temperature of a sample of accurately known weight, as well as the direction and degree of enthalpy changes due to the processes taking place in the sample, and the rate of weight change. By means of the DTG curve, which illustrates the rate of weight loss, the beginning and end of the decomposition processes can be read off in an accurate manner, even in the case of partly overlapping reactions, since in the latter case the curves show a minimum or inflexion point.

The behaviour of a number of carbohydrates has been studied earlier in the Derivatograph (LÓRÁNT & BOROS, 1965), but the derivatographic curves have not been compared with the results of the chemical analysis of the residue and of the volatiles, so that the conclusions are not always correct. These authors attempted to deduce quantitative empirical correlations from the

experimental results generally valid for all carbohydrates, and the experimental conditions have been adjusted to this objective.

These investigations were carried out with the Derivatograph to elucidate the chemical and physical process of the thermal decomposition of glucose, including the accompanying heat effects and weight changes. In order to gain a deeper insight into the processes, chemical analyses were performed after cooling the sample in certain intermediate stages of decomposition and the analytical results were compared with the information obtained by means of the Derivatograph. For the collection of the volatile decomposition products a special device was constructed in which heat treatment could be conducted under conditions similar to those in the Derivatograph.

1. Materials and methods

1.1. Materials

For the derivatographic tests analytical grade glucose (Reanal) was used. For the sake of comparison some intermediate products of caramel formation were also investigated:

5-Hydroxymethyl-furfural (Fluka Co.)
High molecular weight soluble caramel colouring substance.
Preparation: 100 g of glucose was heated in a 150 °C thermostat for 20 minutes, then dissolved in water. The low molecular weight fractions were removed by 72 hours dialysis in a cellophane bag and the residue evaporated to dryness.
Water insoluble caramel colouring substances.
Preparation: 100 g of glucose was heated in a 190 °C thermostat for 10 hours, comminuted and the soluble fraction extracted with distilled water. The dried insoluble residue was used for the derivatographic test.

The derivatograms were compared to the derivatogram of a wheat starch sample (polysaccharide) obtained from the Starch Factory.

1.2. Procedure

A Paulik—Paulik—Erdey Derivatograph manufactured by MOM was used for the tests. To ensure identical experimental conditions the sugar and other substances under investigation were mixed with an inert material to avoid the vigorous foaming at the beginning of decomposition which may cause considerable losses. Twice the amount of alumina ignited above 1000 °C, was used as inert substance. In the course of the tests the temperature of the samples was raised linearly from room temperature, at a rate of 6 °C per minute. The DTA and DTG curves were recorded with 1/5 sensitivity. The samples

could be cooled to room temperature within 10 minutes. The derivatograms were plotted by the instrument as a function of time and were then converted in the usual manner into temperature functions.

In order to account for the effect of air the derivatograms were also plotted in nitrogen atmosphere produced by introducing nitrogen gas in the chamber of the test furnace at a rate of 1 litre per minute.

1.3. Investigation of the residue after interruption of heat treatment

For the investigation of the residue the heat treatment was finished, the sample cooled rapidly to room temperature and dissolved in 25 ml of water. The glucose content of the sample was determined by the Willstätter—Schudel method (BROWN & ZERBAN, 1948) and the solubility of the colouring substance was concluded from the colour intensity of the solution and from the colour of the filtered aluminium oxide.

1.4. Investigation of volatile substances

Fig. 1 shows the device for the collection of volatile substances. A sample of the same composition as that tested in the Derivatograph was heated in a glass tube in nitrogen atmosphere changing its temperature at the same rate as in the derivatographic test, at the rate of 6 °C per minute. The volatile substances were collected in absorbers. The first absorber contained, to bind the oxo-compounds, 2,4-dinitro-phenylhydrazine reagent (4 g of 2,4-dinitro-phenylhydrazine in 1 litre of 2 N HCl). The following contained a 0.05 M barium hydroxide solution to bind the formed carbon dioxide, the latter being quantitatively determined by titration of the excess reagent.

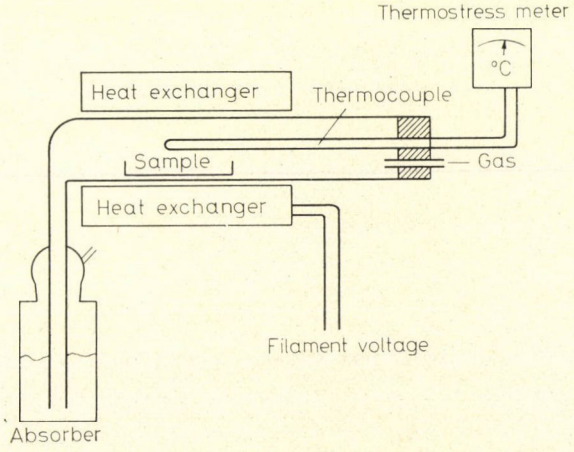

Figure 1. Apparatus for the collection of volatiles liberated in the course of heat treatment

For the continuous recording of the amount of absorbed oxo-compounds the absorber was mounted in place of the cuvette of a single cell photometer (manufactured by MOM) and the amount of oxo-compounds characterized by the light absorption of the insoluble dinitro-phenylhydrazone precipitates in blue light and read together with the temperature of the sample.

In other experiments the absorber was exchanged in every temperature range for fresh reagent containing absorbers.

For the determination of the total quantity of bound oxo-compounds the excess of the 2,4-dinitro-phenylhydrazine reagent was determined iodometrically. To the absorber, containing 20.00 ml of the reagent, 20.00 ml of 0.1 N iodine solution was added and after a reaction time of 5 minutes the excess iodine titrated in the presence of starch indicator solution with 0.1 N sodium thiosulphate. The amount of the bound oxo-compound was calculated from the consumed reagent assuming a molecular weight of 100.

2. Results

2.1. The derivatogram of glucose

Fig. 2 shows the derivatogram of the heat treatment of a mixture of 500 mg of glucose and 1 g of alumina. The minimum at 165 °C on the DTA curve, representing the enthalpy changes, indicates the endothermic heat effect of the melting of sugar. It appears of the TG curve, representing weight changes, that no change occurs in the weight of the sugar up to its melting.

Simultaneously with its melting the decomposition of glucose begins and three phases of the reaction can be distinguished:

The first phase in sugar decomposition begins simultaneously with its melting and ends at 240 °C. This phase is accompanied by an endothermic heat effect approximately identical with the melting heat (about 7.5 kcal per mol) and the weight change amounts to 25 % of the initial weight of the sample.

The second phase of decomposition begins at 240 °C and continues to about 400 °C. In the presence of air this phase begins with an exothermic reaction, followed by a high endothermic peak on the DTA curve at 340 °C indicating an endothermic process superimposed on the exothermic process. Total weight loss up to 400 °C is 68 %.

The third phase of decomposition continues from 400 °C up to the total combustion of the residue. This phase is characterized by an almost linear weight change accompanied by a significant exothermic heat effect and implies the combustion of the residue. The rate of combustion is determined by the diffusion rate of oxygen needed for combustion.

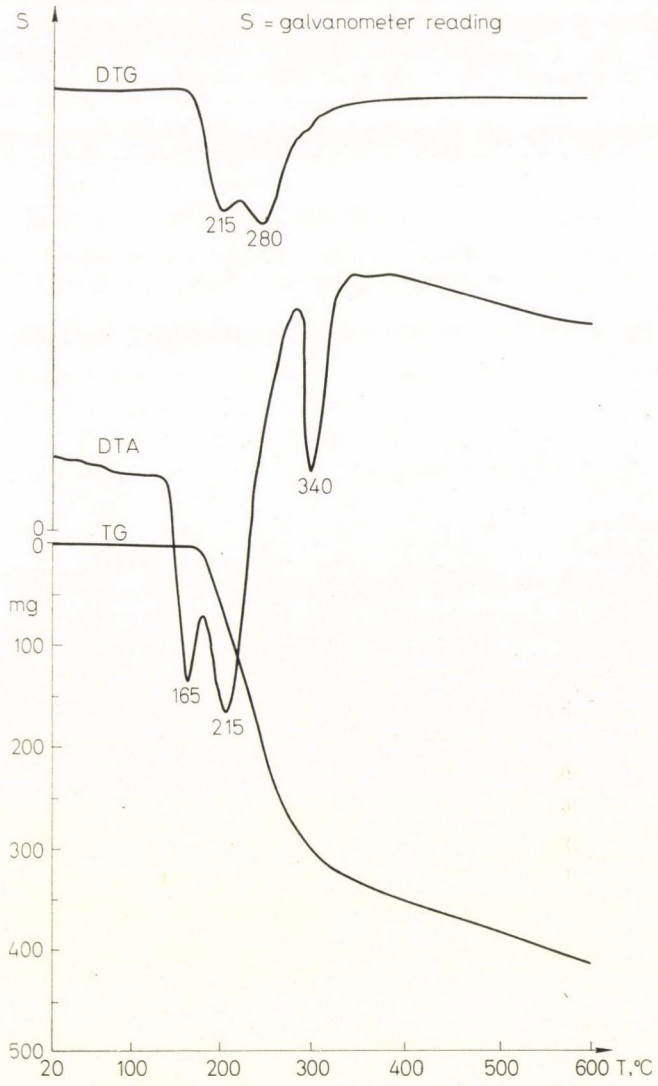

Figure 2. Derivatogram of glucose. (A mixture of 1 g of alumina and 500 mg of glucose heated at a rate of 6 °C per min.)

The curves determined in nitrogen atmosphere in order to eliminate the effect of air, but otherwise under identical conditions, are presented in Fig. 3. The sections of the curves between 20° and 240 °C correspond to the same section of the curves determined in air. There is no weight loss up to 165 °C, the melting point of glucose; herefrom up to 240 °C the weight loss is 25 % in a single step. The second phase between 240° and 400 °C is purely of endothermic nature in inert gas atmosphere and the temperature charac-

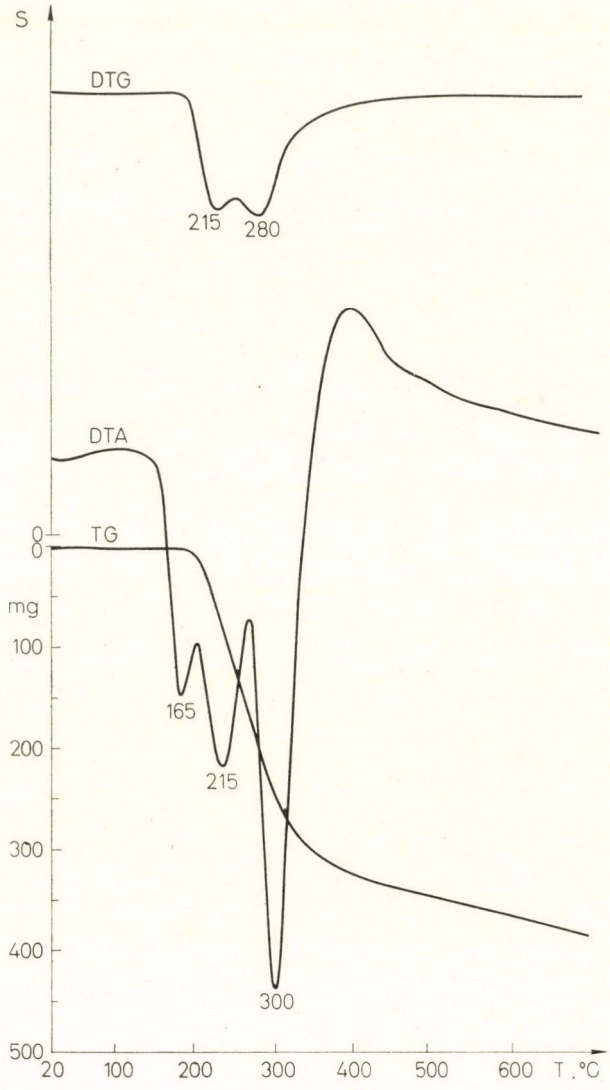

Figure 3. Derivatogram of glucose in nitrogen atmosphere. (A mixture of 1 g of alumina and 500 mg of glucose heated at a rate of 6 °C per min.)

teristic of the process is 300 °C. In the course of the process weight loss rises to 68 %, indicating that the processes in the inert gas atmosphere are the same as those observed in the presence of air, but the decomposition products formed in air are ignited and the exothermic heat effect is caused by the heat resulting from combustion.

The third phase of decomposition is exothermic in inert gas atmosphere, too, and the weight of the sample decreases almost linearly with time. When

the heat treatment was interrupted at 600 °C nothing but some fine carbon powder mixed with alumina remained in the crucible. The quantity of the residue was about 25% of the initial sample which was considerably less than the carbon content (40%) of the untreated glucose sample indicating the formation and evaporation of significant amounts of volatile carbon compounds.

For a closer study of these changes the volatiles and residues formed in the various phases of heat treatment were subjected to investigation.

2.2. Investigation of volatile substances

The volatile substances, evaporating directly from the sample, cannot be investigated in the Derivatograph, since the air space of the latter is not completely closed, though it can be continuously ventilated with some inert

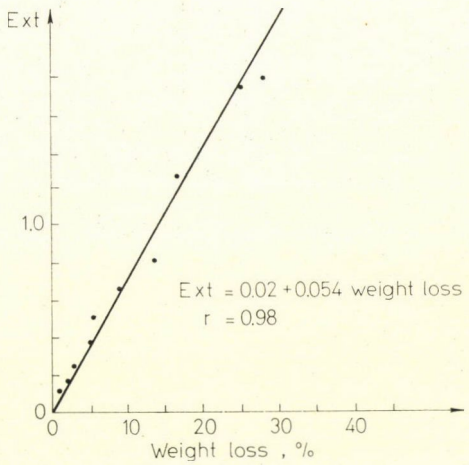

Figure 4. Quantity of oxo-compounds formed during heat treatment of glucose vs. total volatiles

gas or with air. The volatile substances formed at the high temperature of decomposition can be led from the apparatus only with the help of an about 20 cm long rubber tubing which, because of the condensation of the products, does not allow for quantitative removal and absorption. For this reason the volatiles were investigated in a specially constructed device. A close correlation was found between the total quantity of oxo-compounds formed and bound in the absorber and the weight loss recorded by the Derivatograph, as shown in Fig. 4. In this Figure the quantity of oxo-compounds is presented by means of the light absorption of the insoluble 2,4-dinitro-phenylhydrazones

in the absorber, while the weight loss is the value measured in the Derivatograph at sample temperature read simultaneously with the photometer. This correlation is linear within a wide range indicating that the device reproduces satisfactorily the heat treatment conditions applied in the Derivatograph and that the volatiles contain a constant proportion of the oxo-compounds in the decomposition phase roughly up to 200 °C. A deviation from linearity appears only at higher decomposition temperatures.

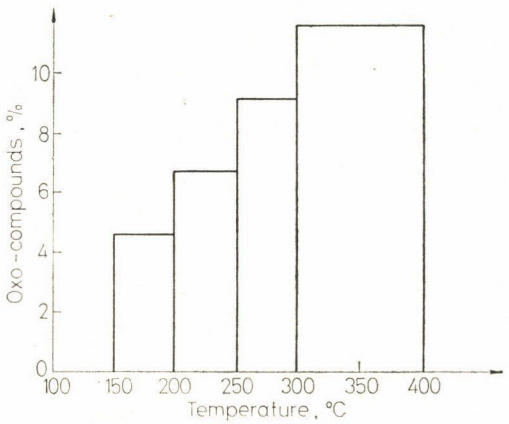

Figure 5. Quantity of oxo-compounds formed from heat treated glucose in various temperature ranges, in percentage of total volatiles

For a more accurate determination of the quantity of 2,4-dinitro-phenylhydrazones, as well as for the investigation of decomposition in a broader temperature range, the absorber was changed at 200 °C, 250 °C and 300 °C and the sample heated up to 400 °C. The excess reagent in the absorbers was determined iodometrically and the quantity of bound oxo-compounds calculated. In Fig. 5 the quantity of bound oxo-compounds in each temperature range (calculated with an average molecular weight of 100) is expressed as percentage of the weight loss found in the Derivatograph in the same corresponding temperature range.

5 to 10 % of the volatiles was bound in the form of 2,4-dinitro-phenylhydrazone in the absorber, and this quantity rose to more than 10 % only at temperatures above 300 °C, indicating a vigorous decomposition process at these temperatures.

Formation of carbon dioxide was observed only at temperatures above 240 °C. The quantity of bound carbon dioxide was about 0.5 mol per mol of glucose.

2.3. Investigation of the residue

The results of the investigation of the residue after cooling in various phases of derivatographic heat treatment are presented in Fig. 6.

In this Figure the results are plotted vs. the maximum temperature prior to cooling and the values of weight losses read from the derivatogram are also shown. It appears that in the course of heat treatment the quantity

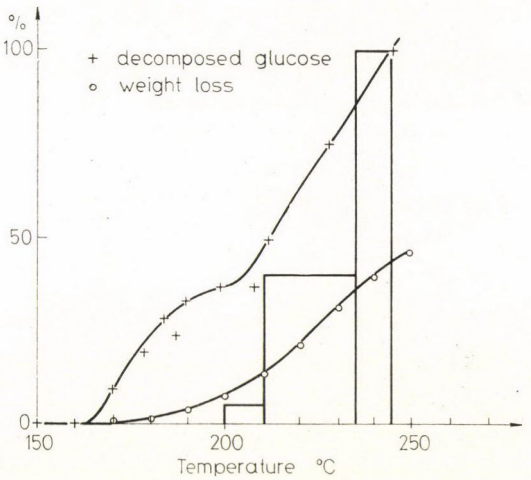

Figure 6. Quantity of decomposed glucose and the weight loss measured in the Derivatograph vs. temperature

of glucose drops rapidly to about 60 %, reaching this value at around 200 °C, while the weight loss hardly exceeds 10 %. In this phase, as confirmed by chemical analysis (TELEGDY KOVÁTS, ÖRSI & VÉGHELY, 1966) the main reaction is the formation of reversion products.

The quantity of reversion products reaches an equilibrium value, while less than 10 % of water can evaporate. (If 100 % of the glucose were converted into polymer, then 1 mol glucose would yield 1 mol (10 %) of water.) A weight loss of over 10 % is due to decomposition accompanying the formation of the reversion product and associated with the formation and evaporation of the oxo-compounds.

When heat treatment is continued the total quantity of glucose is converted into an insoluble brown matter and volatile products. Thus the formation of caramel takes place in the first phase, up to 240 °C, as observed in the Derivatograph and the later phases relate merely to the decomposition and pyrolysis, or, in inert gas atmosphere, to the carbonization of the formed caramel.

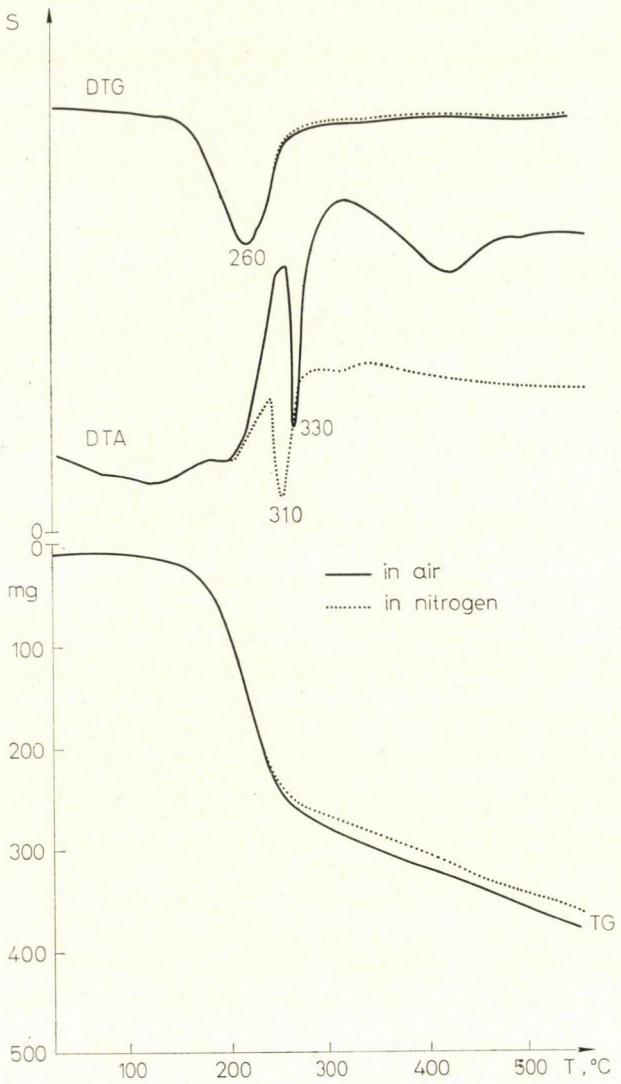

Figure 7. Derivatogram of 5-hydroxy-methylfurfural. (A mixture of 1 g alumina and 500 mg of 5-hydroxy-methylfurfural heated at a rate of 6 °C per min. in air and in nitrogen.)

2.4. Derivatographic investigation of other substances

The above conclusions were supported and supplemented with further details by the derivatograms of some available intermediate products. These derivatograms, plotted under identical conditions with that of glucose, are presented in Figs 7, 8, 9 and 10.

It appears from the derivatograms of the soluble and insoluble caramel colouring matter and of the wheat starch (Figs 8, 9 and 10) that in the tem-

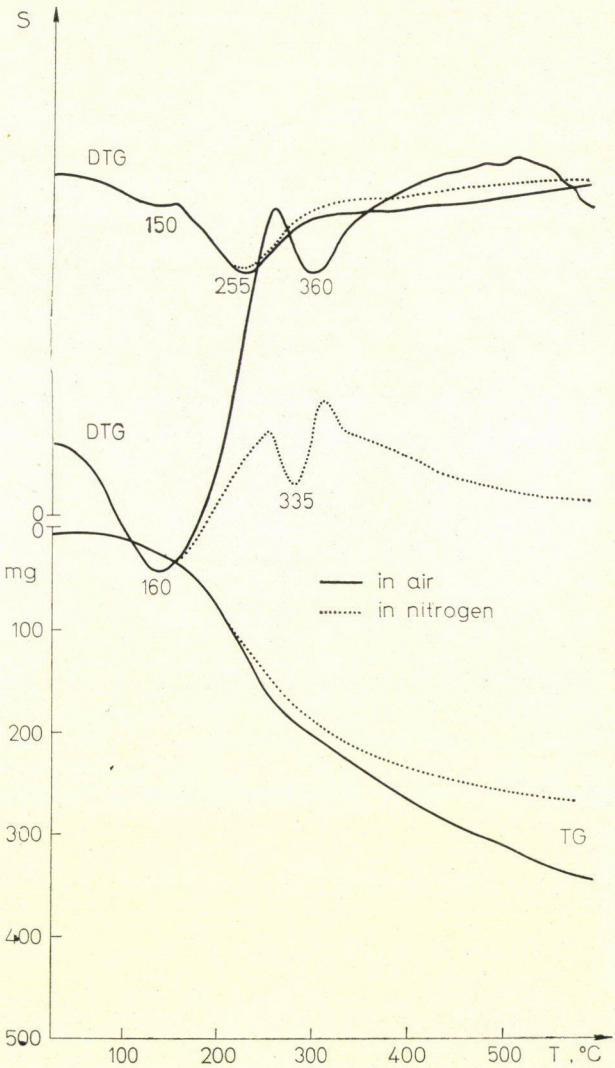

Figure 8. Derivatogram of the soluble caramel. (A mixture of 1 g of alumina and 500 mg of soluble caramel heated at a rate of 6 °C per min in air and in nitrogen.)

perature range between 20 and 200 °C an endothermic process takes place indicating the escape of moisture from the samples. In the case of 5-hydroxy-methylfurfural (Fig. 7) no such process can be observed, since crystalline 5-hydroxy-methylfurfural contains no water.

According to several authors 5-hydroxy-methylfurfural is the colourless intermediate in the formation of caramel. In fact the derivatogram in Fig. 7 shows at 260 °C a significant (up to 50%) weight loss which, however, is too high to be accounted for by the decomposition reaction alone. It is more

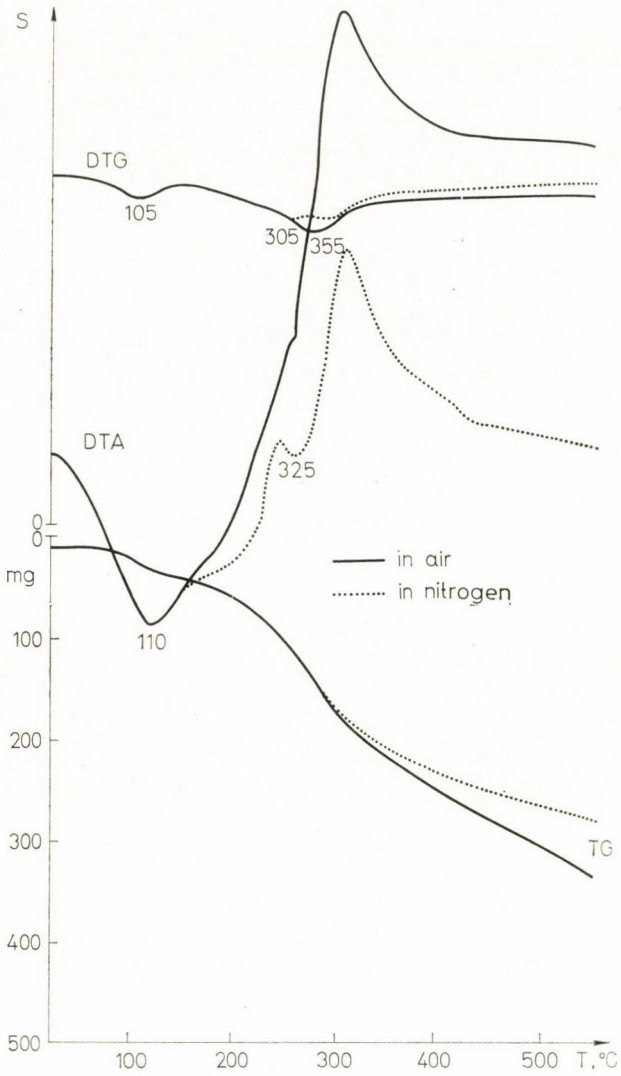

Figure 9. Derivatogram of the insoluble caramel. (A mixture of alumina and 500 mg of insoluble caramel heated at a rate of 6 °C per min in air and in nitrogen.)

probable that only part of the 5-hydroxy-methylfurfural is converted into colouring matter, while another part evaporizes at this temperature and will appear among the products volatilizing above 200 °C. The rest of the 5-hydroxy-methylfurfural is converted into colouring matter and will pyrolyze and burn giving a curve similar to that observed for the combustion of caramel.

The derivatograms shown in Figs 8 and 9 have much in common indicating identical or similar processes. There is, however, a significant difference with respect to the temperature at which decomposition begins. The soluble

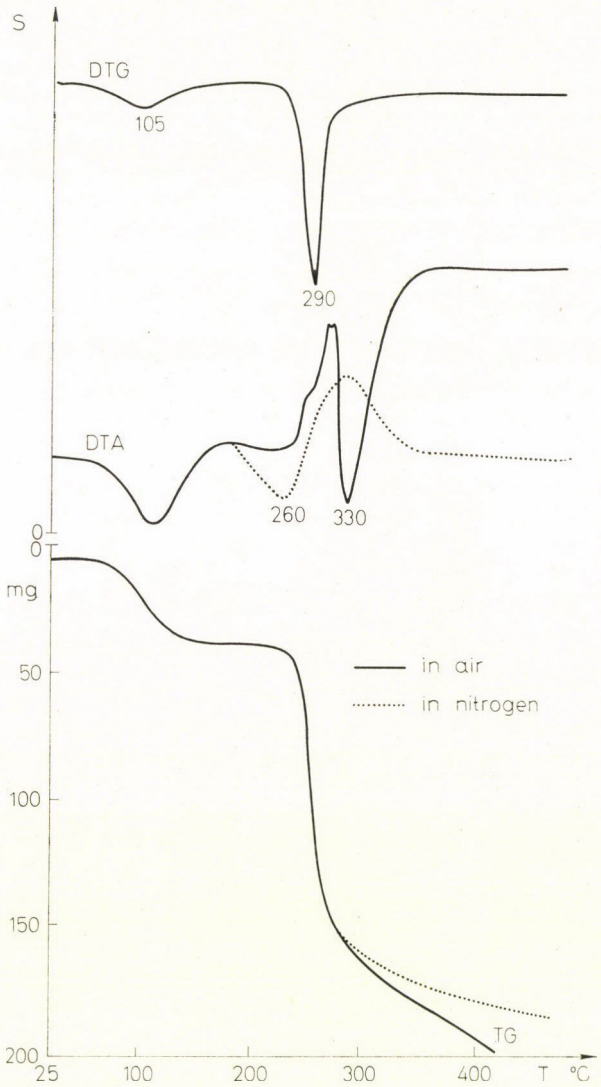

Figure 10. Derivatogram of wheat starch. (A mixture of 1 g of alumina and 250 mg of wheat starch heated at a rate of 6 °C per min in air and in nitrogen.)

high molecular weight colouring matter begins to decompose at 235 °C, while the insoluble colouring matter decomposes only at temperatures above 305 °C. This observation supports the assumption that in the temperature range below 240 °C the glucose is transformed into caramel, between 240° and 300 °C further decomposition and formation of insoluble colouring substance occurs, while above 300 °C the insoluble colouring substance carbonizes.

The section above 200 °C on the wheat starch derivatogram shown in Fig. 10 is surprisingly similar to the corresponding section of the derivatogram

of glucose. This similarity suggests the formation of reversion products (polysaccharides) in the course of caramel formation and these reversion polysaccharides contribute to the development of the characteristic decomposition pattern.

3. Conclusions

On the basis of these experiments the derivatograms of glucose (Figs 2 and 3) may be interpreted as follows:

Decomposition begins with the melting of sugar; upon reaching 240 °C it decomposes under the formation of volatile and non-volatile products. Above 200 °C the volatiles contain 5-hydroxy-methylfurfural, too.

At 240 °C the brown caramel colouring matter is no longer soluble in water. Beside it, or bound to it reversion products (polysaccharides) are also present. In the temperature range between 240 °C and 400 °C the caramel and the reversion products decompose in the course of endothermic reactions and the decomposition products ignite in air.

Further heat treatment of the residue in air results in combustion, in inert gas atmosphere in carbonization.

It follows from the results of these experiments that when investigating the mechanism and kinetics of caramel formation temperatures above 240 °C must not be applied for any length of time, since this may lead to the thermal decomposition of the caramel colouring substances and the great number of decomposition products and the chemical changes will confuse the results and make evaluation impossible.

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MEASUREMENT OF WATER ACTIVITY OF BIOLOGICAL SUBSTANCES AND INTRODUCTION OF THE CONCEPT OF STERIC "HYDRATURE SURFACE"

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The importance of hydrature (ERH) of biological substances and its role in the food and feed industries are discussed. A simple and inexpensive apparatus for the determination of ERH, easily set up in any laboratory, is described.

Tomato concentrate was chosen as the model substance and its dehydration by the foam mat drying method was investigated.

The authors suggest a novel method for the study of hydrature in a three dimensional coordinate system. From the shape of the hydrature surface further information can be gained for the characterisation of different materials. The moisture content of the material, X, is plotted perpendicularly to the enthalpy-concentration chart of the humid air and in this steric coordinate system a surface will correspond to the saturation and another to the equilibrium state. This enables a more complex interpretation of storage and drying phenomena. Drying processes take place in the space above the ERH surface, while humidification will occur in the space bordered by the ERH and saturation surfaces. In this way it will be possible to perform calculations which shall be described in a later communication.

It is a well known fact that in the majority of cases the vapour pressure of solutions is lower than that of the pure solvent. While in the case of well defined solutions of known concentration this reduction of the vapour pressure can be determined by calculation from Raoult's law, in case of complex substances, such as substances of biological origin, this can be determined only by experiment.

This property of the biological substances is due partly to the fact that in the cell juices a great variety of substances are dissolved in concentrations varying between wide limits, partly to certain physiological parameters, such as maturity, time and place of growth, etc. Because of these complex conditions vapour pressure reduction cannot be determined by calculation, not even when the water content is known. The reduced vapour pressure of biological substances, that is their equilibrium relative humidity (ERH) derived from the former, as well as ERH/100, known as water activity (a_w) is a function of hydratation state or hydrature of the substance.

Hydrature consists of the following elements:

— monomolecular or BET (Brunauer—Emmett—Teller) layer;
— adsorbed water and
— "free" water.

Naturally, in case of biological substances there is no true "free" water, since in fact this, too, is a solution, just as the BET layer has to be considered also from the aspect of hydrophilic surfaces and their charge patterns in the given material.

In the case of biological substances the sorption isotherms are of sigmoid character, as reported by BALLSCHMIETER (1967), VAN TWISK (1969) and many others (Fig. 1).

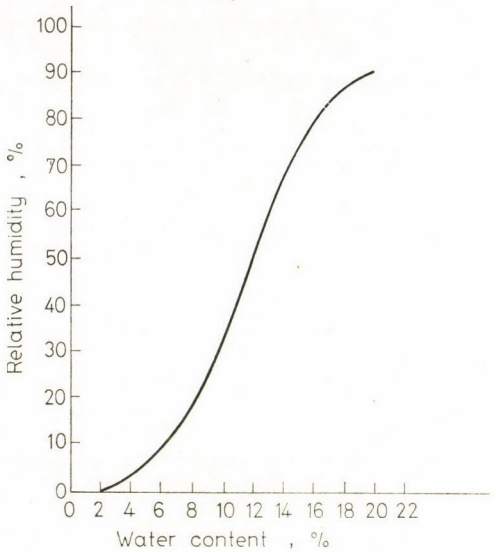

Figure 1. Sorption isotherm of corn flour according to van Twisk

According to ROCKLAND (1969) the sorption isotherms consist of three separate local isotherms: i.e. LI-I, LI-II and LI-III, each one belonging to a hydrature element, as mentioned above.

These local isotherms are shown in Fig. 2.

ROCKLAND (1969) draws far-reaching conclusions from this theory. He claims that the material in state LI-I, when the BET layer is incomplete, tends to form free radicals which show poor resistance to chemical oxidative deterioration. The water content of biological substances which can be characterized by the LI-III range, is high enough to be liable to attack by microorganisms. The biological substances in state LI-II can be stored satisfactorily. Hydrature can also be studied by means of the determination of the ERH or a_w of biological substances. The various methods used for the determination of ERH have been described by JOHN and SEKHON (1968).

It is generally known that microorganisms can display vital activities only above a certain relative humidity threshold (in this respect, however,

there are differences between the species, varieties and individuals), which implies, that they are capable of living only on biological materials which possess water activities above this threshold value. The dependence of the vital functions of bacteria on water activity as well as that of all living organisms, may be traced back to the ERH dependence of enzymatic and other purely chemical reactions which form the basis of vital processes. ACKER (1969) has demonstrated that the lipolytic enzyme reaction of neutral lipids

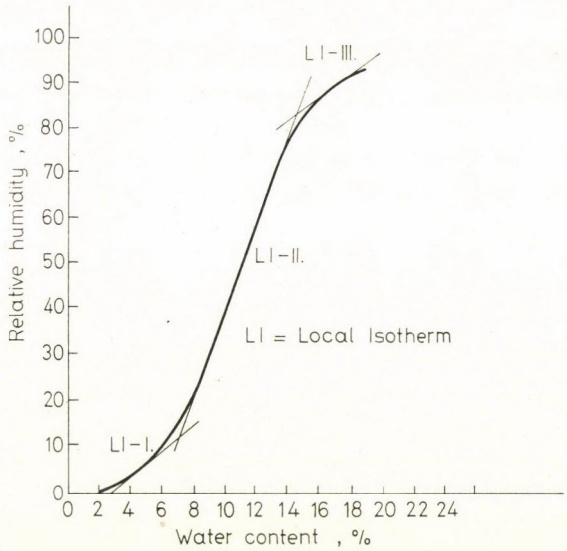

Figure 2. Sorption isotherm of corn flour according to van Twisk as interpreted by Rockland

is inhibited below a certain ERH level. While studying the hydrolysis of pectin LENGES et al. (1969) have found that enzymic pectin hydrolysis does not occur below $a_w = 0.8$, and chemical pectin hydrolysis is inhibited below $a_w = 0.4$.

In the knowledge of water activity, it will be possible to create biotic, anabiotic or even abiotic conditions and this possibility is actually made use of in the food industries, in fermentation, storage and drying processes, respectively.

The hydrature of biological substances was studied by means of sorption tests or ERH measurements. Generally, in sorption measurements the temperature is kept constant, preferably in a thermostat, the substance under investigation is introduced into the air space of known relative humidity, formed above various salt solutions and the weight change, i.e. the change in the water content of the sample is determined. In the ERH tests a large quan-

tity of the substance under investigation is introduced into a relatively small volume of air and the relative humidity of the air above the substance is determined at various temperatures, while the water content of the substance remains relatively constant.

From the aforesaid it follows that both test methods investigate the same phenomenon from two different aspects.

At a given pressure the hydrature of a substance is determined by three parameters: the moisture content of the substance, the temperature and humidity of the ambient air. These parameters are interlinked and if a change is brought about in the value of any of the parameters, this will involve a modification in the values of the other two parameters. The proportion of the changes is a function of the properties of the substance under investigation, however, this will be discussed later.

We suggest the plotting of water activity in a steric coordinate system where the three axes stand for the above three parameters. In such a coordinate system the actual hydrature conditions can be characterized by a single point.

If the temperature and the water content of the substance change, — and this is usually the case during processing and storage of food products—, the point characteristic of the hydrature of the substance will move on a special surface in space. We have further assumed that this surface is characteristic of the substance under investigation, and some useful practical information can be gained from the shape of this surface. The following paragraphs contain a report of the work done relative to this subject.

1. Materials and methods

1.1. Tomato concentrate

All experiments were carried out with samples taken from a 5 kg jar of tomato concentrate, manufactured by the Canning Factory, Dunakeszi, in 1969 and having a refractive index of 28.6 %.

1.2. Experimental drying equipment

The tray type drying equipment was built in the workshop of the Institute, the device was heated by means of electric power and the air temperature was controled.

Ventilator motors: 200 V, 150 W, 9000 rpm.

Heating cartridges: 2×1200 W.

Tray dimensions: outside dimensions 306×138 mm; perforated part: 292×123 mm; perforated holes: 3.175 W mm diameter; spacing: 4.76×4.76

mm, in quadratic arrangement; height of the edge, round the perforated area: about 3 mm.

The axonometric diagram of this experimental drying equipment is shown in Fig. 3 and the drying tray in Fig. 4.

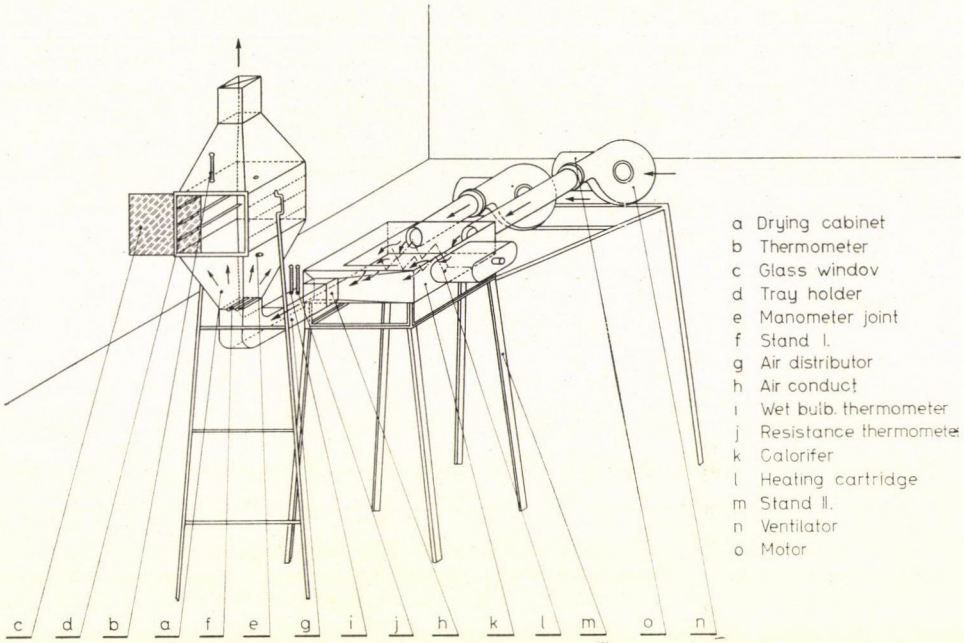

Figure 3. Laboratory foam drier

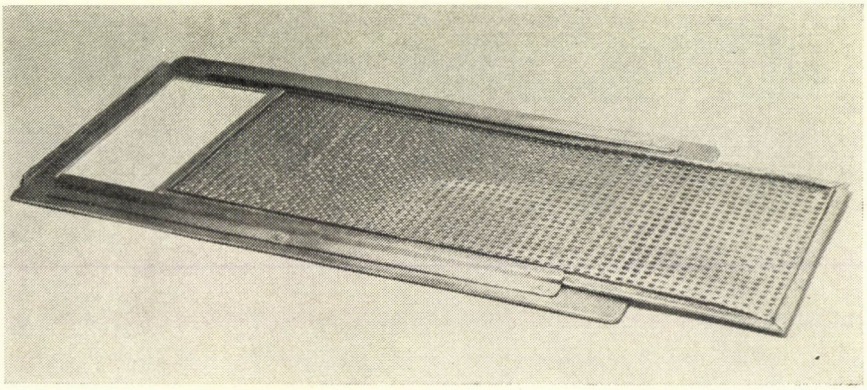

Figure 4. Perforated drying tray, framed

1.3. Equipment for measuring equilibrium relative humidity

The ERH apparatus based on the measurement of the conductivity of lithium chloride was built in our workshop. It is shown in Figs 5 and 6.

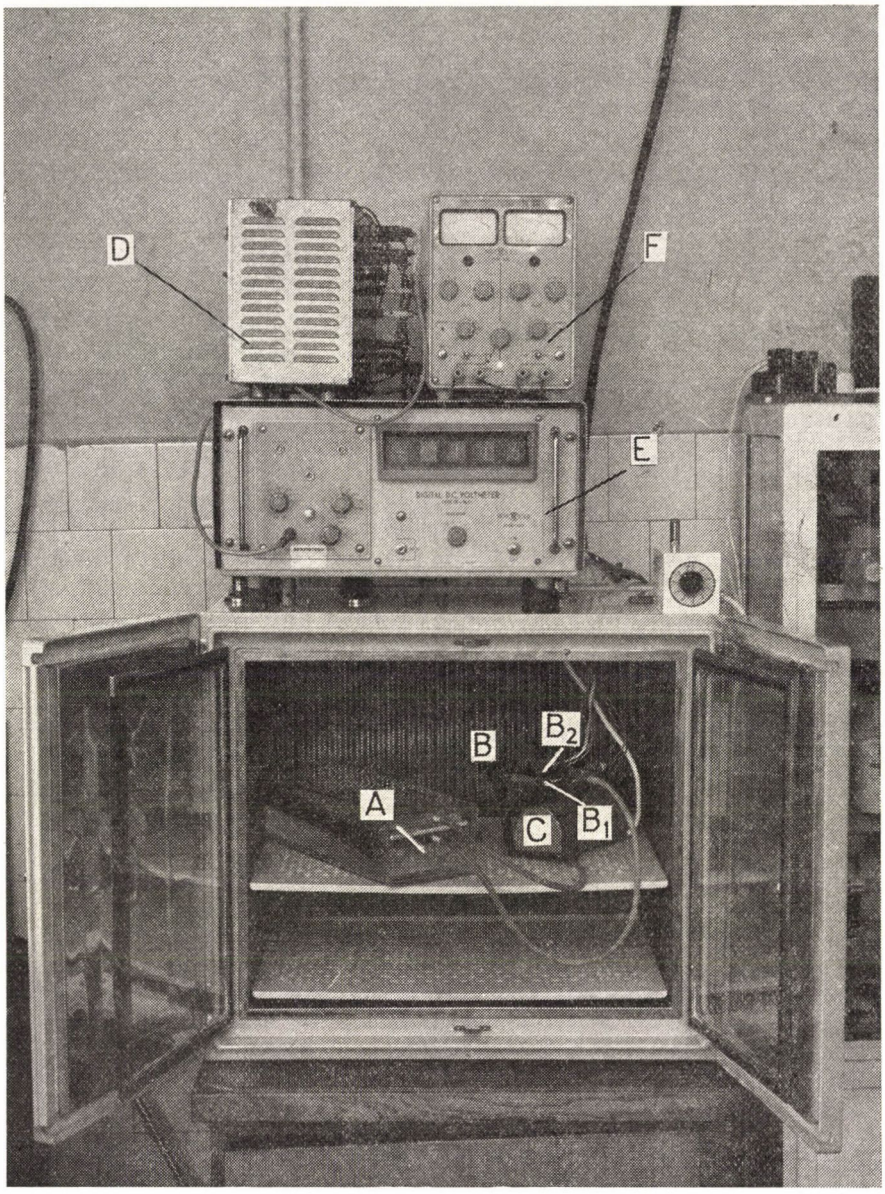

Figure 5. Apparatus for the determination of ERH

The principle of the apparatus is illustrated in the diagram of Fig. 7.

The ERH apparatus consists of the following parts:

Measuring chamber (A) a 36 ×13 ×10 cm glass tank covered with an acrylic glass plate. To reduce the air space within the chamber a 5 cm thick acrylic glass block is placed into it. The cover is fixed with a rubber band to

the tank and gas tight closing is ensured by rubber packing. An aluminium plate frame carries the perforated tray in the chamber. The cover is provided with 2 pipe outlets for connection to the rubber tubing in which the gas circulates.

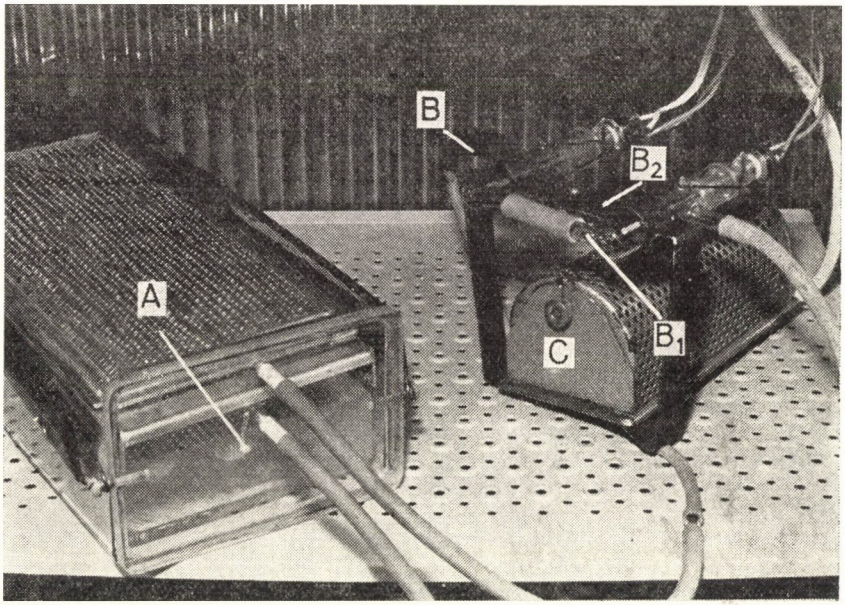

Figure 6. The thermostated units of the apparatus for the determination of ERH

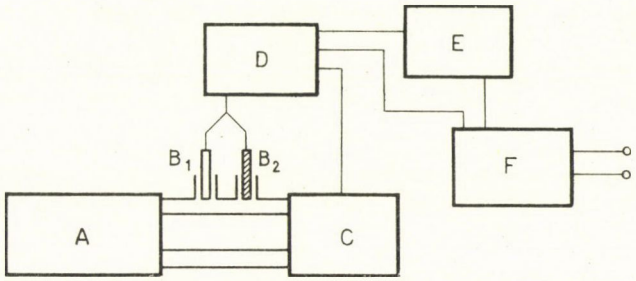

Figure 7. Schematic diagram of the apparatus for the determination of ERH

Probes (B). Two probes are inserted into the circulating air stream (B-1 and B-2), one of them is an electric resistance thermometer, the other one a LiCl sensor.

Membrane pump (C) provides for gas circulation. Its capacity is 2 litre per minute.

Measuring bridge and supply unit (D) for measuring the electric signals and to supply current to the membrane pump and to the probes. Depending on the position of the switch of the unit the signals coming either from the resistance thermometer or from the LiCl sensor reach the bridge.

Digital tube voltmeter (E) for reading the voltage on the bridge. Type EMG-1361, manufactured by Orion, factory for electric equipment.

Direct current supply unit (F) type EMG-1852, manufactured by Orion factory for electric equipment. This unit supplied 12 V stabilized D.C. to the measuring bridge.

A laboratory thermostat, type LP 102, manufactured by "Labor" was used to stabilize the temperature in the ERH-meter.

1.4. Refractometer

Type Model II, manufactured by Zeiss, Jena.

1.5. Drying

The tomato concentrate is applied to the drying tray with the help of a palette and planed with an appropriately shaped metal plate so as to fill evenly the 1/8 inch (3.18 mm) high frame of the tray. The tray is then transferred into the drying equipment described in para. 1.2, and exposed to air of 100 °C temperature. Special cratering nozzles were not used, but by partial choking of the air stream (by partly covering the perforations of the tray) such high local levels of air velocities were achieved that cratering occurred spontaneously at the majority of holes. The drying experiments were carried out for definite periods (10, 20 and 30 minutes).

1.6. Determination of equilibrium relative humidity

The original tomato purée samples, as well as the samples dried for different periods were placed, with the drying tray, into the measuring chamber of the ERH-meter.

The measuring chamber was closed with the cover and fixed by means of the rubber band. By switching on the electric units the circulation of air was started and at the same time the electronic measuring devices were charged. The constant voltage values both for the lithium chloride sensor and the resistance thermometer were measured on the digital tube voltmeter. These values were recorded and the temperatures measured by the resistance thermometer and the LiCl sensor were determined by means of the curves of HENGSTENBERG and co-workers (1957). In the knowledge of temperatures, the vapour pressure values pertaining to the measured temperatures were read from the curves published by HENGSTENBERG and co-workers (1957).

Calculation:

$$ERH = \frac{P_{LiCl}}{P_{H_2O}} \times 100 \quad (1)$$

where

ERH = the equilibrium relative humidity, %
P_{LiCl} = saturation pressure above the lithium chloride sensor, mm
P_{H_2O} = water vapour pressure pertaining to the given temperature, mm

ERH was measured in three series of experiments at different temperatures (30, 50 and 70 °C). The required temperature was obtained by the adjustment of the thermostat. The ERH value belonging to each treatment (tomato puree dried for a certain time and at a certain temperature) was measured by four parallel measurements.

Prior to the measurements the resistance thermometer and the lithium chloride sensor were calibrated at various temperatures, with salt solutions of known ERH value.

1.7. Determination of the refractive index

The refractive index of the original tomato concentrate was determined in the usual manner by placing some tomato purée on a piece of filter cloth and allowing some drops of the juice to fall on the measuring prism by twisting the cloth.

In the case of dried tomato concentrate this method could not be directly applied, therefore the re-dilution method suggested by Vas and co-workers (1958) was used as described below.

After the determination of the ERH a total of 100 g of the dried product was taken from different places of the tray and weighed on a laboratory counterbalance. To this sample exactly 30 g of distilled water was added and the material homogenized by stirring, followed by the determination of the refractive index. In the calculation of the results the degree of dilution was accounted for. The referactive index was determined after the measurement of ERH because the air circulating during the ERH determination caused a modification in the sample when equilibrium relative humidity was reached and the measured value referred to this modified water content.

2. Results

The ERH of the original tomato concentrate (para. 1.1) and of dried products prepared from the former was determined at three different temperatures in four replicates.

Since the initial material itself was slightly inhomogeneous (a minor separation of the serum was observed), though it was homogenized by stirring prior to its application to the tray, and the extent of crater formation during drying on the tray was also different in each drying experiment, significant differences were found in the refractive indices of the samples dried for equal periods. Changes in the refractive index caused of course significant deviations in the ERH values.

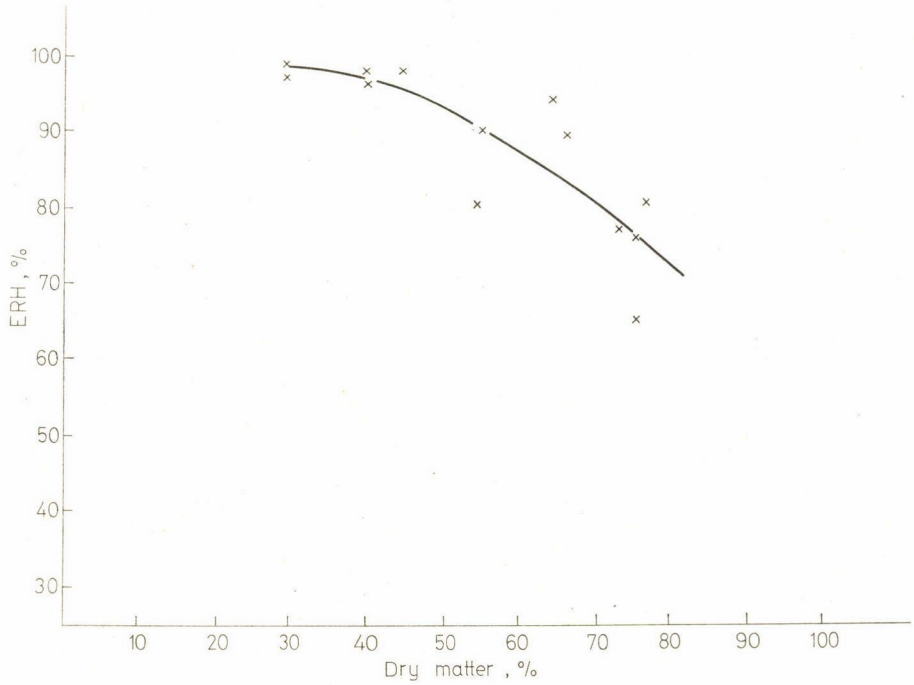

Figure 8. ERH values of tomato concentrates at 30 °C

We have, therefore, evaluated our experiments by plotting the ERH values and the pertinent refractive indices of a series measured at the same temperature on the same diagram (16 measurements). In this way from each series of measurements a single curve was obtained. The curves are shown in Figs 8, 9 and 10.

It appears from the Figures that the ERH curves represent functions of higher order. By means of a coordinate transformation the logarithms of the related moisture contents and ERH values were obtained, leading to the following regression equations:

$$\log \mathrm{ERH}_{30\,^{\circ}\mathrm{C}} = 1.531 + 0.256 \log \mathrm{X} \qquad (2)$$

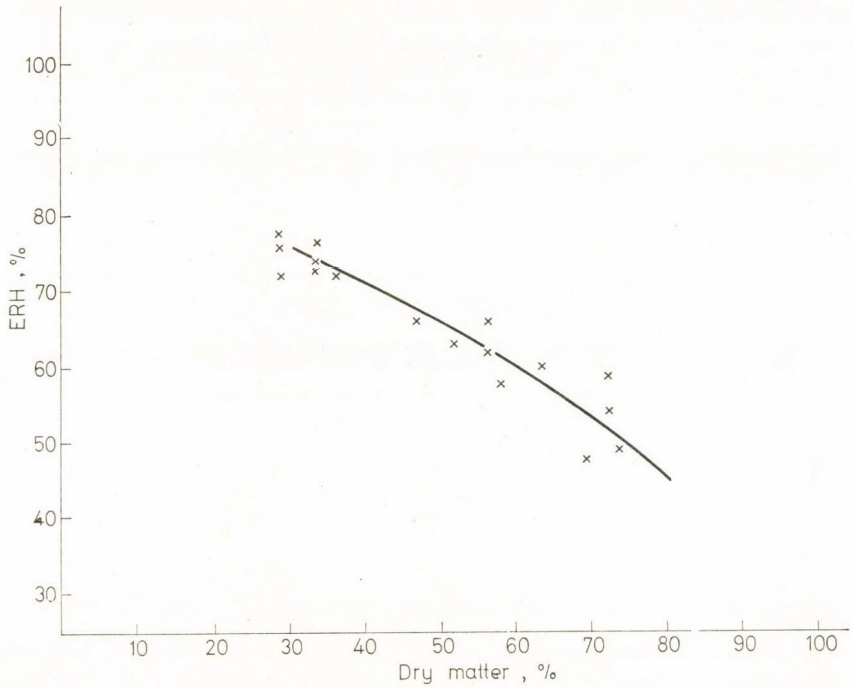

Figure 9. ERH of tomato concentrates at 50 °C

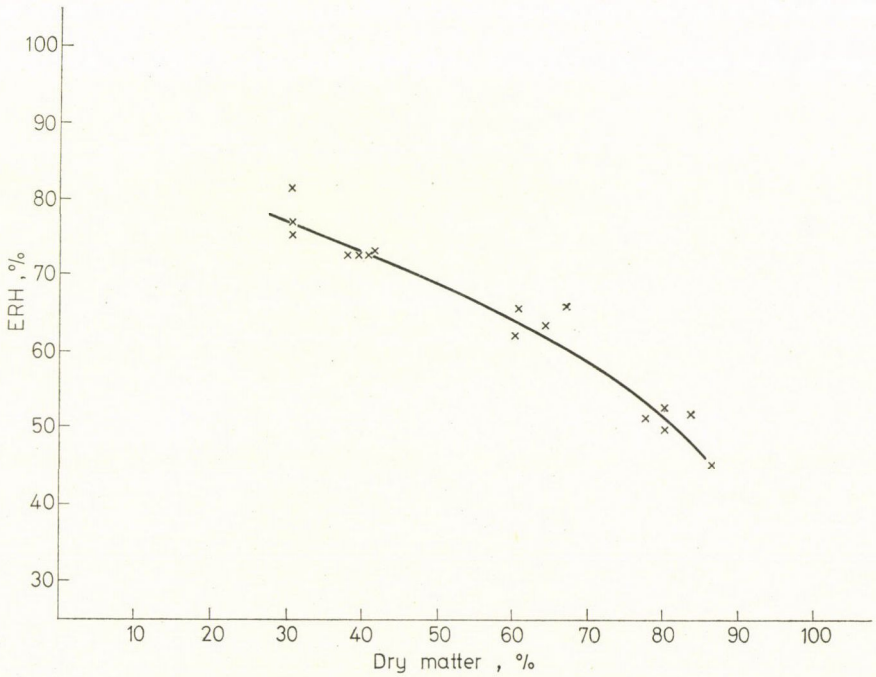

Figure 10. ERH of tomato concentrates at 70 °C

$$\log \mathrm{ERH}_{50\,^{\circ}\mathrm{C}} = -0.006 + 0.997 \log \mathrm{X} \tag{3}$$

$$\log \mathrm{ERH}_{70\,^{\circ}\mathrm{C}} = 1.318 + 0.3167 \log \mathrm{X} \tag{4}$$

The following correlations were found between the logarithms of the series of pairs of values:

$$r_{30\,^{\circ}\mathrm{C}} = 0.840 \tag{5}$$

$$r_{50\,^{\circ}\mathrm{C}} = 0.989 \tag{6}$$

$$r_{70\,^{\circ}\mathrm{C}} = 0.928 \tag{7}$$

where

X = moisture content, per cent
r = correlation coefficient.

From these curves the ERH values of tomato concentrates showing refractive indices of 30, 40, 50, 60, 70 and 80% respectively, were determined at 30, 50 and 70 °C. The values obtained in this manner were plotted on the enthalpy-concentration chart of humid air so that the three values corresponding to each refractive index were plotted on a separate diagram. The saturation curve $\varphi = 1$ is also shown on each diagram leading to a total of 6 Molier diagrams. If these diagrams are specially arranged by plotting a third (X) axis on which the moisture content is shown, a steric coordinate system is obtained in which the ERH values form a surface. The ERH surface is shown axonometrically in Fig. 11.

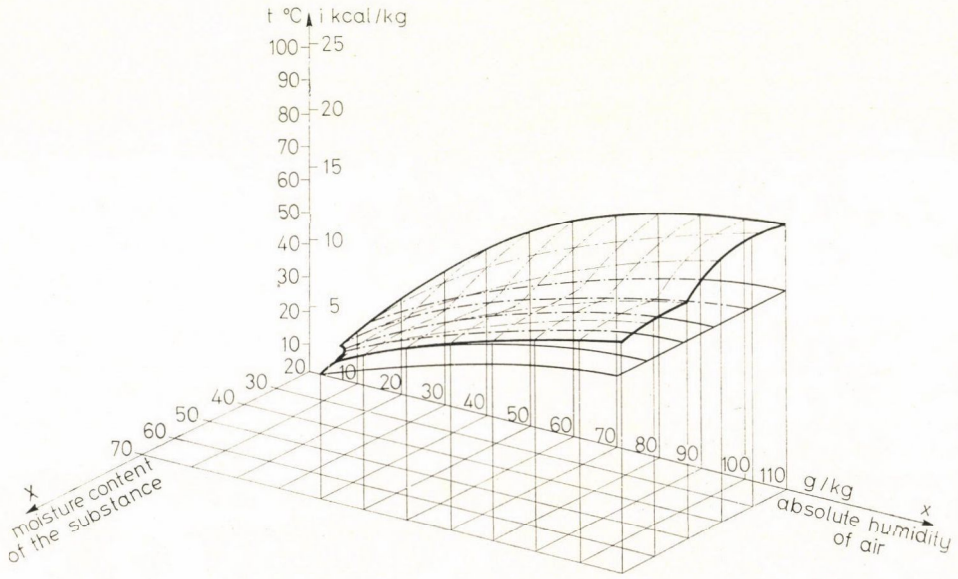

Figure 11. The ERH surface of tomato puree

The symbols used in the text in accordance with the enthalpy-concentration chart are:

x = absolute humidity of air, kg/kg
φ = relative humidity of air, %
i = enthalpy, kcal/kg
X = moisture content of the material, % (as refractive index)

It appears from the Figure that the ERH surface has flexions in various directions of the space.

It appears further that the edge of the plotted surface is near to the saturation curve in case of low refractive indices (at high water content), while at low water content it deviates from the $\varphi = 1$ lines.

Another flexion can also be observed. The ERH surface is not parallel to the saturation surface, but forms a "vault" above it.

3. Conclusions

As a result of our experiments we have arrived at a peculiarly shaped ERH surface by plotting ERH in a steric coordinate system, achieving thereby a synthesis of the ERH and sorption test methods.

From the aspect of steric geometry the plotted equilibrium surface is a surface of higher order on which the number of flexions corresponds to the order. The surface can be approximated, section by section, by planes and the intersection lines of these approximating planes will indicate changes in the nature of hydrature, just as the sections approximated by straight lines in case of the ROCKLAND isotherms. Thus, for instance, the rupture of the BET layer may manifest itself on this surface in the form of an inflexion or torsion, just as thermal coagulation at high temperatures.

The ERH surface of tomato was numerically described by means of a matrix in which the first column is the moisture content of the material, the first line the absolute humidity of air and the other data, as a function of the two related parameters, are the temperatures which determine the surface at any given point (Table 1).

The shape of the hydrature surface can be interpreted only on the basis of a large number of manifold tests. We believe that beyond the axonometric illustration and the matrix type description, as applied in this paper, the shape of the hydrature surface might also be interpreted with the help of certain mathematical, or three dimensional geometrical equations. We intend to continue our experiments in this direction.

Surface plotting has, however, predictable application potentialities. There is a hygroscopic phase between the ERH surface and the saturation surface.

Table 1

Matrix of the ERH surface of tomato puree

X	Absolute humidity of air, kg/kg				
	20	40	60	80	100
20	30	46	62	68	70
30	24	46	58	67	71
40	24	42	54	63	69
50	24	38	48	55	64
60	24	38	48	55	60
70	24	38	48	54	60

X = moisture content of the material, %

The space above the ERH surface contains the conditions characteristic of drying.

The shape of the hydrature surface permits of certain conclusions concerning the hygroscopic properties of the material. The more distant the surface from the $\varphi = 1$ surface, the more hygroscopic will be the material. Hygroscopicity is on the other hand negligible when the ERH surface fits the saturation surface.

By means of steric plotting of the ERH surface it will be possible to get some information for the design of drying equipment. The number of contact stages or transfer units can be determined by some of the known methods. For instance, the graphic calculation which is known as the McCabe—Thiele method can be illustrated by steric plotting as follows: The curve of the working line is plotted, from any of the terminal points of this curve the equilibrium surface can be reached along the straight line in the plane of the i-lines. The slope of this straight line in the direction of the moisture content of the material is determined by the ratio of the gas-solid mass velocities. From this surface in the direction of the working line we reach the plane of material moisture, that is from the thrust point of the first construction line perpendicularly to plane $\varphi = 1$.

In practice the efficiency of contact stages or transfer units must also be considered. Since the efficiency of contact stages is also expressed by the approximation of the equilibrium state from some point of the working line, this method can be extended to the determination of the number of not only the theoretical but the actual stages.

This method offers a possibility of determining the band length or the number of sections in drying equipments for direct, counter or cross streams.

These constructions can be replaced by calculations using the appropriate three dimensional geometrical equations. The details of this procedure will be published in a forthcoming paper.

The authors wish to thank their colleague, Mr. József Somlói for the construction and calibration of the ERH equipment.

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FREEZING WITH LIQUID NITROGEN; EFFECT OF FREEZING RATE ON THE QUALITY OF SLICED BEEF

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Freezing experiments with 2 cm thick raw beef cuts (longissimus dorsi) were aimed, on the one hand, at the comparison of liquid nitrogen spray freezing with air blast freezing and, on the other, at the investigation of the effect of freezing rate on meat quality.

Liquid nitrogen spray freezing was performed in a small tunnel system experimental equipment. The slices of meat were frozen first by reducing their temperature to −1 °C by the introduction of cold nitrogen gas and subsequently adjusting the final temperature to −30 °C by spraying liquid nitrogen on the surface of the product.

For air blast freezing, too, a small tunnel system experimental equipment was used in which the air temperature was −40 °C and the flow rate of air was 1.8 m/sec. The final freezing temperature of the meat slices was the same as in the experiment with liquid nitrogen, namely −30 °C.

Average linear freezing rate (W; core temperature from 0° to −5 °C) was 15 cm /hour in the case of liquid nitrogen spray freezing and 4 cm/hour in the case of air blast freezing.

The results of the experiments, intended to compare the effects of freezing rate on meat quality, have shown that weight loss during freezing was significantly lower when liquid nitrogen spray freezing was used (at a 15 cm/hour average linear freezing rate) than in the case of air blast freezing (at 4 cm/hour average linear freezing rate).

No significant difference was found in thawing drip losses within the applied average linear freezing rate range of 4 to 15 cm/hour, while the loss on cooking of the meat frozen by the liquid nitrogen process was somewhat higher than of the meat processed by the other method. The total weight loss during freezing, thawing and cooking was practically the same in the range of 4 to 15 cm/hour average linear freezing rates.

According to the results of instrumental measurements after thawing the colour of meat frozen by the liquid nitrogen method was somewhat paler, but this difference was not perceptible to the naked eye.

There was no significant difference in the texture and eating quality of the meat slices frozen by the two different methods.

In the past ten years the application of liquid nitrogen to the freezing of foods has spread everywhere in the world. According to CLOUGH (1969) there are about 120 liquid nitrogen freezing plants in the world today.

Of the three types of technologies, namely: immersion into liquid nitrogen, spraying with liquid nitrogen and nitrogen gas processes, in recent years the spraying technique has gained ground in Europe, first of all in France, the United Kingdom, West Germany, Belgium and the Netherlands.

Freezing plants operating on the principle of spraying with liquid nitrogen are continuously operating horizontally placed freezing tunnels provided

with conveyor belt, circulating ventilators and nitrogen spraying nozzles. The freezing tunnel consists of three zones: precooling zone (with nitrogen gas), freezing zone (spraying with liquid nitrogen) and equilibration zone (with nitrogen gas). The food on the conveyor belt travels continuously at an appropriate speed through the three sections (precooling, freezing, equilibration zones). The final temperature of the product can be adjusted by the speed of travel and by the quantity of sprayed nitrogen (PLACZEK, 1969; DINGLINDER, 1969; CLOUGH & DARLINGTON, 1969; BRITISH OXYGEN COMPANY, BRENNAN *et al.*, 1969).

The effect of the high freezing rate, achieved by means of the liquid nitrogen process, on the quality of food has been studied by a great number of investigators.

MOISEVA and PISKAREV (1959) found a lower thawing drip loss and a higher cooking loss in sliced beef frozen by the liquid nitrogen process than in sliced meat frozen slowly. No difference was observed in the organoleptic properties of fresh (not frozen) meat slices and those frozen in liquid nitrogen.

KONDRUP and BOLDT (1960) have reviewed and evaluated the international literature on meat freezing from 1935 onwards. They conclude that the picture presented by this literature is not clear on the degree to which the eating quality of meat is affected by freezing rate, though the bulk of the publications stresses that freezing rate has, beyond all doubt, an effect on the quality of meat and that this effect appears after thawing and processing of the meat.

According to several authors freezing rate has a measurable effect on the hardness of meat and on thawing drip loss, while only a minor change appears in the eating quality. They claim that a comparison of the data found in the literature is difficult, since in most cases detailed information on the thickness of meat slices, on the applied freezing rate and time are lacking.

They point out that the following two essential questions are still awaiting to be answered:

Will the effect of freezing rate on the quality of meat still be apparent after a given storage period?

What is the lowest permissible freezing rate and the freezing rate which will produce the best quality product?

ASTRÖM and LÖNDHAL (1969) studied the quality of sliced beef frozen by means of the liquid nitrogen and the air blast processes. (The authors refrain from describing the method of liquid nitrogen freezing, but the 8 minutes freezing period permits of concluding that they have used the spray method.)

According to their experimental results the weight loss during freezing of sliced meat frozen by the liquid nitrogen method is higher, its thawing drip loss and cooking loss lower than of meat frozen by means of the air blast method.

The eating quality of sliced meat frozen by liquid nitrogen process is better than that of meat frozen by air blast process.

BENGTSSON and JAKOBSSON (1969) performed the freezing of sliced beef by immersion into liquid nitrogen, by spraying with liquid nitrogen and by the air blast method. They found that weight loss during freezing of sliced meat is lower when any of the two methods using liquid nitrogen is applied compared to the loss caused by the air blast method, but the difference between the weight losses is considerably less than that cited in the literature. Thawing drip loss is the same for all three freezing methods, but the cooking loss of meat frozen by immersion into liquid nitrogen is higher than of meats frozen by spraying with liquid nitrogen or by the air blast method.

Sensory testing has shown that with increasing freezing rate the colour of the meat becomes paler. This is not noticeable in the eating quality of the products.

In this paper the results of the experiments aimed at the comparison of the methods of spraying with liquid nitrogen and freezing by air blast, and at the investigation of the effect of freezing rate on the quality of sliced beef will be described.

1. Materials and methods

1.1. Materials

In the freezing experiments sliced beef (longissimus dorsi) was used; each experiment was carried out in two replicates.

The meat used in the first freezing experiment was the meat of young cow beef, in the second experiment of younger beef animal.

The thickness of the slices was 2 cm, their weight was between 170 and 200 g.

The slices were frozen without preliminary cooking or packing.

1.2. Equipment

Freezing experiments were performed by means of the liquid nitrogen spraying system and the air blast process.

1.2.1. Equipment for spraying with liquid nitrogen. The equipment consisted of a 150 cm long, 40 cm wide and 30 cm high insulated tunnel.

The perforated tube for the introduction of nitrogen and for spraying liquid nitrogen was placed on the upper wall of the tunnel, along its whole length.

Freezing was performed by first cooling the meat slices to —1 °C by introducing cold nitrogen gas, subsequently spraying liquid nitrogen on the surface of the product thereby freezing all meat in the tunnel simultaneously to a final temperature of —30 °C.

1.2.2. Air blast freezing equipment. The apparatus consisted of a tunnel of the same dimensions as used in the liquid nitrogen freezing equipment. The freezing tunnel was placed into a cold room of controlled temperature. Cold air was circulated by a ventilator placed at the entrance of the tunnel.

The temperature of the air was —40 °C, its flow rate 1.8 m/sec.

The final temperature of the frozen meat slices was the same as that of liquid nitrogen frozen meat, namely —30 °C.

1.3. Analytical methods

1.3.1. Weight loss. Weight loss (due to dehydration) during freezing was determined by weighing the individual slices before and after freezing.

1.3.2. Drip loss. For the determination of drip loss frozen slices of meat of known weights were packed into moisture proof plastic pouches. The meat was allowed to thaw in the pouch in a vertical position.

Thawing temperature: 1 °C
Thawing period: 48 hours.

Loss due to leakage was determined from the weight of the juice collected in the bag.

1.3.3. Colour. The colour of the samples was determined before and after freezing, using Gardner's Colour Difference Meter. The colour of the surface of the sample in direct contact with liquid nitrogen or air during freezing and the colour of the surface of freshly cut slices were examined. To thaw the samples they were kept prior to testing on air for one hour at 20 °C.

1.3.4. Texture. The texture of the samples was determined before and after freezing using the Instron apparatus and accessory equipment. Before the experiment the slices of longissimus dorsi were weighed and then sealed under vacuum in polythene bags. The bags were immersed in a constant temperature water bath of 80 °C for 60 minutes. The bags were cooled by running water of 15 °C for 2 hours. Finally the slices were reweighed.

After heat treatment 1 ×1 ×2 cm sections of the longissimus dorsi with fibres parallel to the 2 cm edge were compressed between 1 cm Wolodkewitsch type jaws.

The samples were examined at 20 °C.

1.3.5. Electron microscopy examination. The samples were subjected to examination before and after freezing in order to determine any eventual effect of freezing on the length of the sarcomeres.

1.3.6. Cooking loss. The frozen meat slices were packed into aluminium foil bags. After thawing the slices were heat treated at 180 °C for one hour. Loss on cooking was determined from the weight loss suffered during heat treatment.

1.3.7. Sensory evaluation. The sensory evaluation of cooked meat was performed by a panel of 10 judges using the triangular taste testing procedure. Appearance, taste, tenderness and juiciness were judged.

2. Results

2.1. Freezing rates and their comparison

The freezing curves of beef slices frozen by liquid nitrogen spray and by the air blast method, respectively, are shown in Fig. 1.

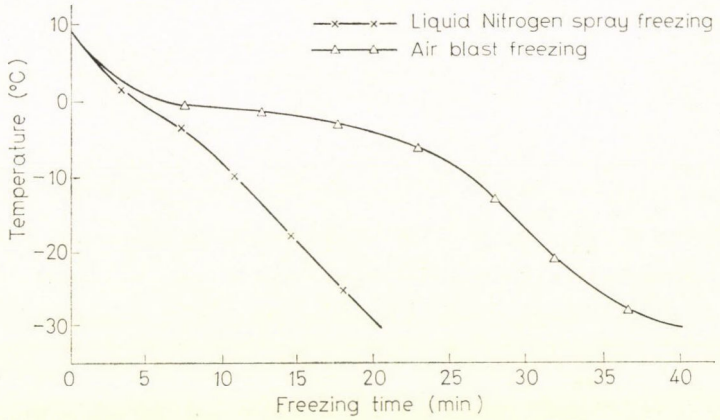

Figure 1. Comparison of freezing rates. The freezing curves of beef slices (longissimus dorsi) using liquid nitrogen spray and air blast freezing, resp.

From the freezing curves (Fig. 1) for core temperature vs. time the following average linear freezing rates were obtained. (W = temperature of the core between 0° and —5 °C.)

Liquid nitrogen freezing (W_1): 15 cm/hour.

Air blast freezing (W_2): 4 cm/hour.

By means of liquid nitrogen spray method an about four times higher average linear freezing rate was achieved than by air blast freezing.

2.2. Effect of freezing rate on the quality of frozen sliced beef

In the course of the first experiment the weight loss due to freezing, drip loss, changes in colour and texture of slices of meat frozen by liquid nitrogen spray and air blast methods, as well as the loss on cooking and any eventual change in the sensory properties of the meat thus treated, were determined.

In the next series of experiments changes in texture and the loss due to leakage caused by freezing, as well as the effect of the freezing rate on the length of the sarcomeres were investigated.

Weight loss, drip and cooking losses are shown in Table 1.

The colour of the slices of beef and its change due to freezing are given in Table 2.

Table 1

Weight loss, drip loss and cooking loss of liquid nitrogen frozen and air blast frozen beef slices (longissimus dorsi)

	Liquid nitrogen frozen II	Air blast frozen III	Statistical significance of differences between averages of treatments II and III, resp.
Weight loss (%)	1.0	1.5	+
Drip loss (%)	3.9	4.3	0
	(4.5)	(4.6)	0
Cooking loss (%)	30.4	29.5	0

Key: ( ) = second experiment
+ = significant ($P \leq 0.05$)
0 = not significant

Table 2

Changes in colour as a result of freezing

Comparison of fresh, liquid nitrogen frozen and air blast frozen beef slices (longissimus dorsi)

	Fresh I	Liquid nitrogen frozen II	Air blast frozen III	Statistical significance of differences between averages of treatments	
				I—II and III, resp.	II and III, resp.
Brightness	26.2	25.3 T	25.2 T	+	0
		25.0 F	25.2 F	+	0
Hue	20.7	24.0 T	21.6 T	+	++
		23.8 F	22.5 F	+	0+
Saturation	20.3	17.8 T	18.7 T	+	+
		18.5 F	18.0 F	+	0
Redness	19.0	16.2 T	17.3 T	+	+
		17.0 F	16.6 F	+	0

Key: T = top surface
F = freshly cut surface
++ = highly significant ($P \leq 0.01$)
+ = significant ($P \leq 0.05$)
0+ = near to the 0.05 level of significance ($P > 0.05$)
0 = not significant ($P > 0.05$)

Table 3 contains the results of the experiments designed to determine the effect of freezing on the texture of the meat and on the length of the sarcomeres, while Table 4 lists the results of the sensory evaluation of cooked slices of beef.

Table 3

Changes in texture and sarcomere length as a result of freezing

Comparison of fresh, liquid nitrogen frozen and air blast frozen beef slices (longissimus dorsi)

	Fresh	Liquid nitrogen frozen	Air blast frozen	Statistical significance of differences between averages of treatments	
	I	II	III	I—II and III, resp.	II and III, resp.
Texture (Toughness, kg for 1 cm Wolodkevitsch jaw)	7.97	4.30	4.06	+	0
	(2.66)	(1.89)	(1.84)	+	0
Sarcomere length (μm)	(1.60)	(1.62)	(1.53)	0	0

Key: ( ) = second experiment
+ = significant ($P \leq 0.05$)
0 = not significant

Table 4

Taste panel results

Sensory evaluation of liquid nitrogen frozen and air blast frozen cooked beef slices, using the triangular test

	No. of judges	No. of correct answers	Statistical significance	No. of correct answers		
				Preferring nitrogen frozen sample	Preferring air blast frozen sample	Without preference
1. Taste	10	4	0	1	3	0
2. Taste	10	2	0	0	1	1

Key: 0 = not significant ($P > 0.05$)

3. Conclusions

The freezing experiments with 2 cm thick raw beef slices (longissimus dorsi) were aimed partly at the study and comparison of liquid nitrogen spray and air blast freezing systems and partly at the investigation of the effect of freezing rate on meat quality.

The average linear freezing rates used in the experiments were: 4 cm/hour in the case of air blast freezing and 15 cm/hour in liquid nitrogen spraying.

The results of the experiments indicated with respect to the effect of freezing rate on the quality of meat a significantly lower weight loss during freezing when the liquid nitrogen spray method with 15 cm/hour linear freezing rate was applied than that observed with air blast freezing at 4 cm/hour average linear freezing rate.

No significant difference was observed in drip loss caused by 4 cm/hour and by 15 cm/hour average linear freezing rates, respectively, but the loss on cooking of the meat frozen by the liquid nitrogen spray method was somewhat higher. As a matter of fact the total weight loss due to freezing, thawing and cooking was practically the same in the range between 4 and 15 cm/hour average linear freezing rates.

After thawing the colour of the meat frozen by liquid nitrogen spray method was, according to the results of instrumental measurements, slightly paler, but the difference was not perceptible to the naked eye. There was no significant difference in the textures and eating qualities of the meat slices frozen by the two different methods. Freezing had no effect on the length of the sarcomeres.

*

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The author now wishes to express his thanks to Dr. CUTTING, Head of the Bioengineering and Microbiology Department, for his guidance, useful advice and help, and to his staff in the M.R.I. for their assistance in carrying out the experiments.

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EXTENSION OF THE STORAGE LIFE OF PREPARED VEGETABLES BY GAMMA-RADIATION

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Mixed vegetable samples consisting of cleaned and sliced carrots, parsley, kohlrabi, head of celery and curly kale were packed in polyethylene pouches and radiation treated between 5 and 800 krad and stored at 6, 9—12 and 20—25 °C, respectively. During storage the sensory quality and viable cell count were established in comparison to that of samples not treated. A panel of judges investigated the odour of the raw unspoiled samples not irradiated and those irradiated with 300 and 400 krad and the taste of the same samples, only cooked. A Texturometer was used to control the effect of radiation treatment upon the consistency of carrots and parsley and upon the softening of carrots when cooked in salted water.

It was established that the storage life of prepared mixed vegetables could be extended by 2 days when treated with 200—300 krad gamma-radiation (Tables 1, 2, Figs 1, 2). A radiation dose of 200 krad reduced the viable cell count by nearly two orders of magnitude and this difference between the cell count of the radiation treated and untreated samples remained constant, though the cell count increased in both samples during storage (Figs 3, 4, 5).

Of the different vegetables tested celery and parsley were most liable to spoilage and curly kale the least liable. Instrumental testing of the samples showed a significant softening in carrots treated with 200 krad. However, the initial difference in the consistency of irradiated and untreated carrots became equalized during storage because of the more rapid softening of the untreated carrots (Figs 6, 7; Tables 3, 4.).

During the first five minutes of cooking in salted water the irradiated samples softened much more rapidly than the untreated ones, however during further cooking the softening of the untreated samples accelerated and thus after 15 minutes the consistency of the samples became equal (Fig. 8, Table 5). Because of the appearance of off-odour and off-flavour it is not advised to apply doses above 200—300 krad (Figs 9, 10, 11).

Vegetables gain importance as the raw material of the rapidly developing food industry. Their economic importance is increased by the fact that, beside being used directly, they form a valuable raw material of the canning industry. From the nutritional point-of-view they serve as a source of vitamins, mineral salts, various organic acids and a great variety of aroma substances.

Beside the supply of raw and canned vegetables the interest in ready-to-cook vegetables is growing as well. The production on large scale of cleaned vegetables for meat broth in polyethylene bags is limited by their short shelf life. Under the present industrial and marketing conditions the storage life of cleaned vegetables is two days at the maximum. The vegetables in their original state are of a relatively good keeping quality (carrots, parsley, celery,

kohlrabi, curly kale), however, when cleaned and sliced they spoil rapidly even packed in foil and stored at low temperature (10—15 °C). KUPFERSCHMID and ZIEGLER (1968) came to the same result in respect to other vegetables.

The aim of our work was to extend the storage life of cleaned mixed vegetables packed in polyethylene pouches by radiation treatment. The investigations were aimed at:

A. establishing the optimum dose range
B. the effect of radiation treatment upon
   a. storage life,
   b. spoilage,
   c. texture,
   d. cooking time.

1. Materials and methods

1.1. Materials used in the experiments

One polyethylene pouch contained the total quantity of 250 g cleaned mixed vegetables, consisting in general of 4 quarters of carrots, 2—3 quarters of parsley, 1 slice of kohlrabi, 1 slice of celery and 1 leaf of curly kale. The celery and parsley pieces were, prior to packing, soaked in sodium hydrogen sulfite solution, in order to prevent browning. For the cooking test carrots of uniform size were selected.

1.2. Treatment of the material

Radiation treatment of the ready-to-cook vegetables was carried out in the ^{60}Co gamma radiation source of 45 kCi activity, at the experimental plant of the Central Food Research Institute.

To establish the optimum dose range the following doses were applied in the course of the preliminary studies: 0, 5, 10, 20, 40, 80, 100, 200, 400 and 800 krad. The dose rate was 100 krad/hour. 10 pouches were treated at each dose level. In the second series doses of 0, 100, 300, 400 and 500 krad were applied at a dose rate of 200 krad/hour and 20 pouches at each dose level.

In the third series the samples were treated with 0, 200, 300 and 400 krad at a dose rate of 200 krad/hour and 25 pouches at each dose level.

To determine the cooking time 0, and 300 krad were applied at a dose rate of 200 krad/hour and 33 carrots of uniform size.

1.3. Storage of the samples

The samples treated at various dose levels were divided into two lots. Half of the sample was stored at ambient temperature, the other half in cold storage. In the first two series the samples were suspended in a storage space of about 6 °C, in the third series they were kept on the shelf in the cold store of the Institute at 9—12 °C.

1.4. Examination of the samples

All three experimental series were tested for spoilage, whether stored at ambient temperature or in cold store. The viable cell count was established only in the third series. From the samples stored at ambient temperature viable cell count was determined on two, or on four occasions; in each case the moiety of two samples in the pouch was used. From the samples kept in cold store viable cell count was determined on five, or on four occasions using again the moiety of two pouches. The second half of the content of each pouch was used to the texturometer tests. For the organoleptic tests the contents of two pouches were used at each dose level on every occasion.

Every pouch was tested for spoilage, however, since a number of pouches were used for various tests during storage, by the end of the storage period only 5—6 samples per treatment were available. Thus in the tables summarizing the result of spoilage tests, only the behaviour of these pouches was given, because only these could be observed during the whole storage period.

1.4.1. Spoilage tests. All the samples, stored at ambient temperature or in cold storage, were tested every day or every second day for spoilage, taking into consideration the following viewpoints:

— appearance of bacterial colonies and/or white exudate;
— appearance of mould colonies;
— softening (soft, smeary feel upon touch);
— discoloration (visible change of colour);
— appearance of off-odour.

An attempt was made to plot the results of the spoilage tests, therefore the extent of spoilage was marked with numbers. The numbers do not differentiate between different kinds of spoilage, only between the extent, because the former is of no importance in the praxis.

Accordingly the degree of spoilage was marked:

incipient spoilage	1
advanced spoilage	2
thouroughly spoiled	3

On the day of testing the numbers marking the degree of spoilage were added up and the sum was considered the spoilage index and used in the dia-

grammatic plotting. Spoilage index 5 or 6 was considered the critical limit, dependent on the number of samples tested. This limit implies that all the samples belonging to the batch underwent spoilage of some extent, thus the whole batch is unfit to eat.

1.4.2. Viable cell count determination. The moiety of two pouchfuls were used at each dose level so as to obtain a more realistic cell count. The accurately weighed, approximately 250 g of mixed vegetable was homogenized in an ETAMIRA apparatus with the double amount of sterile water containing 0.02% TWEEN 80. Dilution assay technique was used in a 9 + 1 series up to the 8th dilution level. The 9 ml of sterile water did not contain TWEEN 80.

The aerobic total viable cell count was determined by plating technique, using universal agar nutrient medium. To determine the mould count malt slant cultures were used and the positive results were evaluated by the method of the most probable cell counts, using HOSKINS' tables (HOSKINS, 1934).

Inoculum was taken at every dilution level, three replicates each.

1.4.3. Instrumental measurement of the texture. For the instrumental tests a "Texturometer" (Zenken Co., Japan) was used.

Texturometer tests were carried out with carrots and parsley only. The roots were cut into slices of 12 mm thickness. The cogged metal head was used for the tests of raw vegetables and the diagrammes were recorded at 750 mm/minute paper velocity.

In tests on the effect of cooking time a plexy head of 23 mm diameter, and concentrically grooved, was used, recording at 750 mm/minute velocity. The hardness of the samples was characterised by the height of the peaks referred to 1 V instrument voltage.

1.4.4. Softening upon cooking. The carrots of uniform size were cleaned and radiation treated. The cooking tests were carried out two hours after radiation treatment. The carrots were placed in boiling water containing 1.5% salt, and 3 pieces were removed every five minutes during the 50 minutes cooking time. During cooking the pot was covered but no pressure was applied. The cooked pieces were left to cool for a quarter of an hour, then a slice of 12 mm thickness removed from each end and 5 slices of 12 mm thickness were cut from the remaining carrot. The slices were tested as described above.

1.4.5. Sensory tests. The raw vegetable was scored for odour, and the vegetable cooked in water containing 1.5% salt, under approx. 0.5 pressure for 15 minutes for flavour on a 5 point scale, where

5 = excellent
4 = good
3 = fair
2 = poor
1 = very poor

Scoring was carried out by a panel of 7 judges. The scores were ranked and statistically evaluated according to KRAMER (1960). Besides, the scores were averaged at each dose level.

2. Results

2.1. Spoilage tests

The first preliminary experiment to establish the preserving radiation dose range was carried out in the spring of 1970. The effect of the nine dose levels (5, 10, 20, 40, 80, 100, 200, 400, 800 krad) was investigated at two temperature levels in comparison with the untreated sample. The samples were tested every day and hardly any difference was found between the samples exposed to doses below 100 krad and the control whether stored at ambient temperature or in cold store. At room temperature the storage life of the samples treated with 100—200 krad was 3—4 days and the samples treated with 400 and 800, respectively, showed deterioration only on the 6th day. The samples stored in the cold store at about 6 °C, showed no change at all till the 7th day of storage. Visible difference between the treatments was observed on the 9th day. The samples treated with 200, 400 and 800 krad respectively had the best appearance. On the 13th day of storage all the samples except those treated with 800 krad showed signs of spoilage. The first to spoile were parsley and celery, the next carrots and kohlrabi and the leaves of curly kale seemed sound and fresh even at the end of the storage period. In the following experimental series the dose range of 100—500 krad was more closely investigated. The samples were treated with 100, 300, 400 and 500 krad, respectively, and stored at ambient temperature and 6 °C. The results are summarized in Table 1 and Fig. 1.

The table shows the spoilage of the individual vegetable varieties as a function of dose and storage time. The curly kale leaf is not included in the table because no change could be observed on it till the end of the storage period, neither at ambient temperature nor in the cold store.

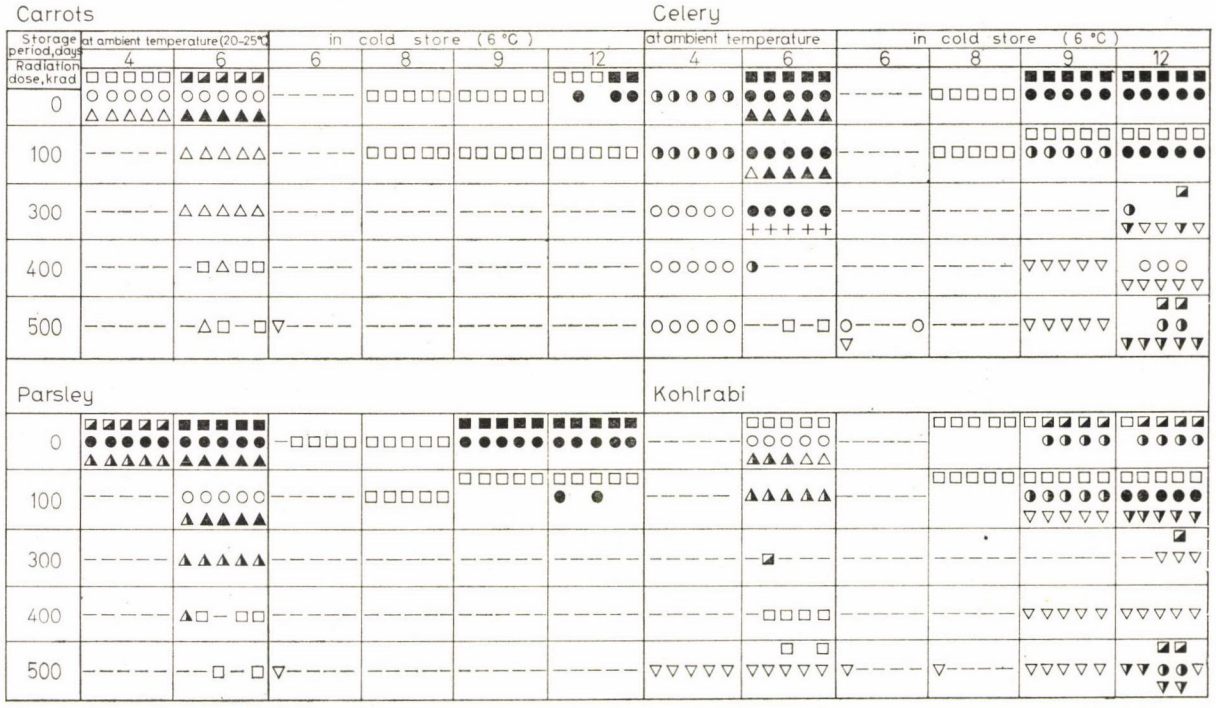
As seen both in the Table and in the diagram the first signs of spoilage at room temperature were observed on the 4th day on carrot and parsley in the control sample, but on every celery sample, whatever treatment it received. On the 6th day of storage the samples treated with 0, 100 and 300 krad were all spoiled and those treated with 400 and 500 krad, respectively, started to deteriorate.

Of the samples kept in cold store deterioration started with the parsley in the untreated sample, on the 6th day of storage.

On the 9th day of storage the untreated sample as well as the one irradiated with 100 krad showed equal signs of spoilage, while in all the other

Table 1

Spoilage of prepared vegetables during storage. Second experimental series



Symbols used:

	Degree of spoilage		
	low	medium	high
Fur of bacteria	□	◪	■
Mould formation	△	◭	▲
Softening, disintegration	○	◑	●
Discoloration	▽	▼	▼

–: sample unchanged

The same samples were tested as each dose level on every occasion. The symbols placed below one another belong to the same pouch, the next one to another pouch.

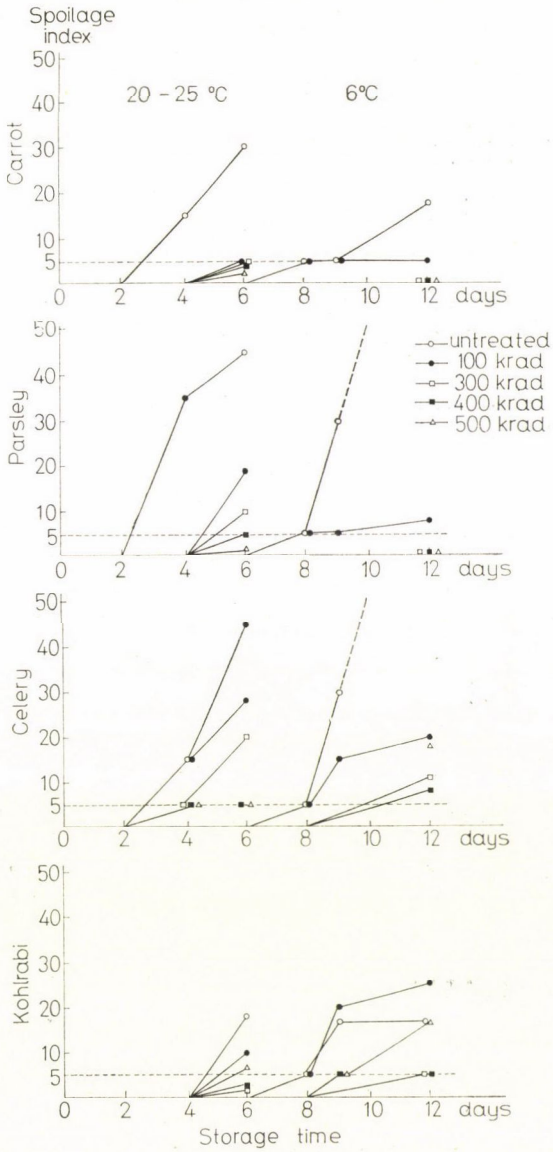
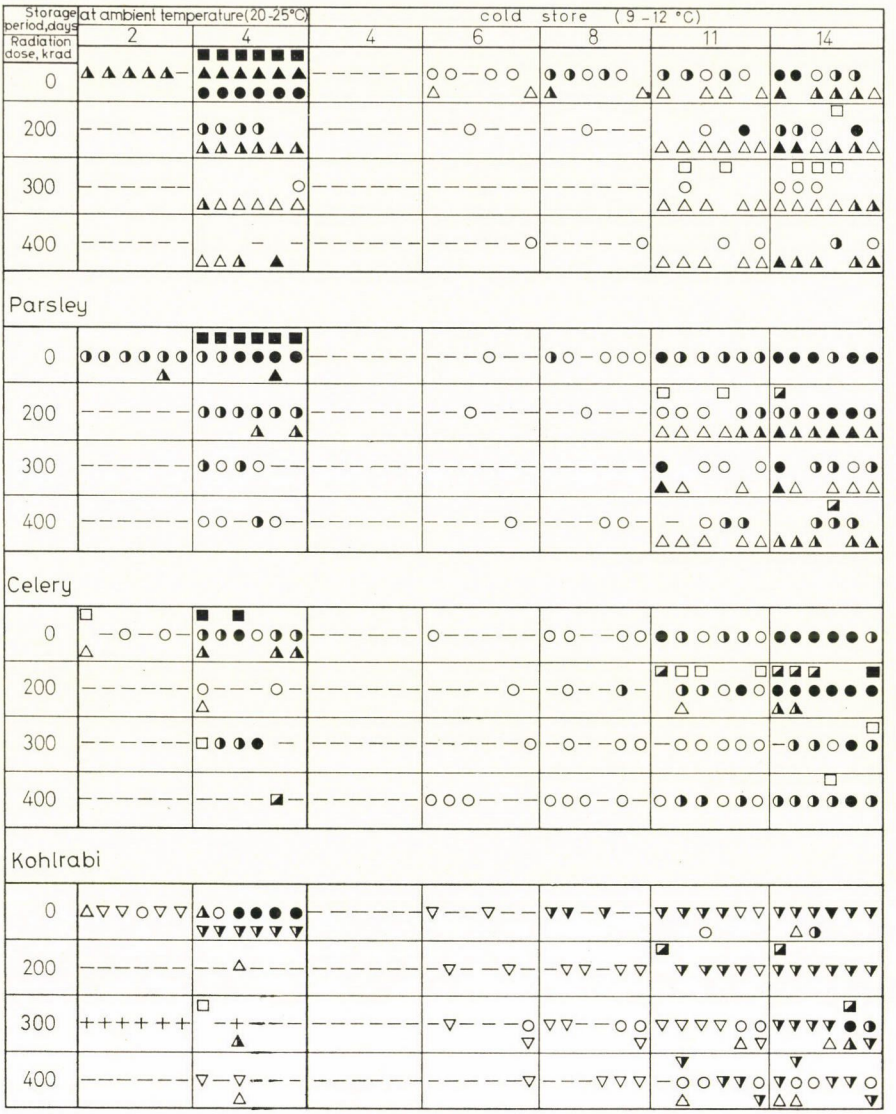


Figure 1. Extent of spoilage of various vegetable varieties as a function of storage time (second experiment). The results of storage at room temperature and in cold store are shown side by side beginning with the last symptom-free day. No difference is made between various kinds of spoilage, the numbers mark their total value. The dashed line indicates the critical limit

samples only the celery and the kohlrabi were slightly discoloured. By the end of the storage period (12 days) all the samples deteriorated more or less. Celery, parsley and kohlrabi were most inclined to spoilage and carrots were the last to deteriorate.

Table 2

Spoilage of prepared vegetable samples during storage. Third experimental series



Symbols used:	Degree of spoilage: low	medium	high
Fur of bacteria	□	◪	■
Mould	△	◭	▲
Softening, disintegration	○	◑	●
Discoloration	▽	▼	▼

–: sample unchanged

On every occasion the same samples were tested at each dose level. The symbols placed below one another belong to the same sample, those next to one another to another sample

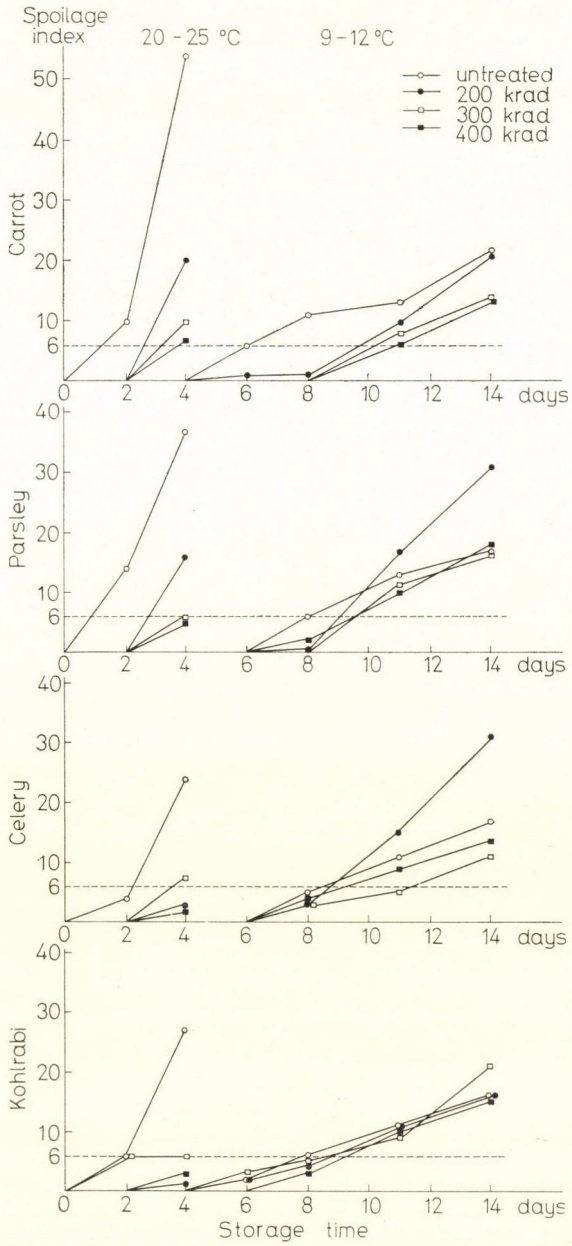


Figure 2. Extent of spoilage of various vegetable varieties as a function of storage time (third experiment). The results of storage at room temperature and in cold store are shown side by side beginning with the last symptom-free day. No difference is made between various kinds of spoilage, the numbers mark their total value. The dashed line indicates the critical limit

In the third experimental series the applied doses were 200, 300 and 400 krad. In this case cold storage was carried out at 9—12 °C. At every dose level the same six samples were tested on every occasion. The results are summarized in Table 2 and Fig. 2.

The untreated samples started to spoile already on the second day of storage, at room temperature. On the 4th day the radiation treated samples started to deteriorate. Parsley and carrots seemed most liable to spoilage, next being celery and kohlrabi. Curly kale leaves remained sound and fresh till the end of the storage period. On the samples kept in cold store the first signs of spoilage appeared on the 6th day. On the 11th day the samples treated at all dose levels were spoiled. Most liable to spoilage were parsley and celery, while kohlrabi turned black to the same extent, whatever treatment it received. On the last day of the storage period (14th day) the curly kale showed signs of average spoilage. An unpleasent off-odour, resembling the smell of fermentation was observed on the 8th day in the untreated samples and in samples treated at different dose levels on the 11th—12th day.

2.2. Viable cell count determination

The viable cell count determinations in the third experimental series are given in Figs 3, 4 and 5.

Fig. 3 shows the viable cell counts of the samples stored at ambient temperature as a function of dose level. Figs 4 and 5 show the cell count of the samples stored in cold store as a function of storage time and dose level, respectively.

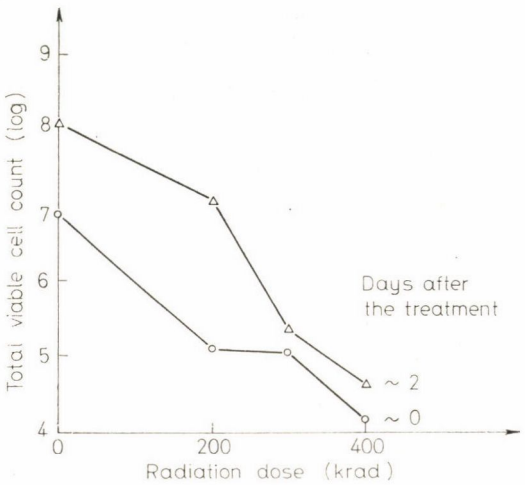


Figure 3. Total viable cell count of the samples stored at room temperature (20—25 °C) as a function of radiation dose (third experiment)

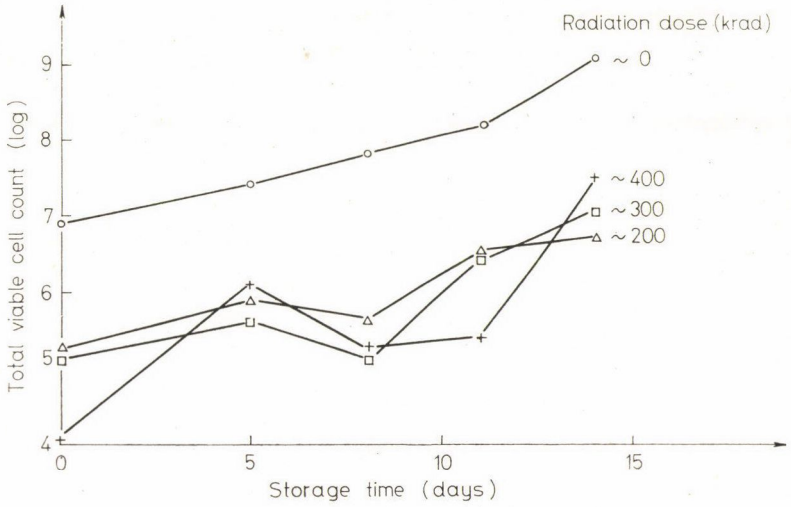


Figure 4. Total viable cell count of the vegetable samples stored in cold store (9—12 °C) as a function of storage time (third experiment)

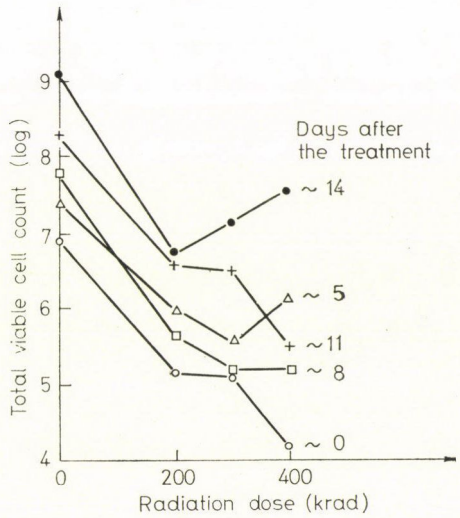


Figure 5. Total viable cell count of the samples in cold store (9—12 °C) as a function of dose level (third experiment)

The highest permitted total cell count for cleaned vegetables consumed after heat treatment is 10^8/g and 10^6/g, if consumed without heat treatment, according to the Hungarian sanitary directives (ORMAY, 1970). The initial cell count of the samples not irradiated was 10^7/g and on the second day reached 10^8/g, if stored at room temperature. The viable cell count in the

samples treated with 200 krad was lower by one order of magnitude, and of those treated with 400 krad with 3.5 orders, than that of the untreated samples. Since on the 4th day of storage the samples treated with 200, 300 and 400 krad, respectively, showed signs of spoilage, they were not tested for viable cell count.

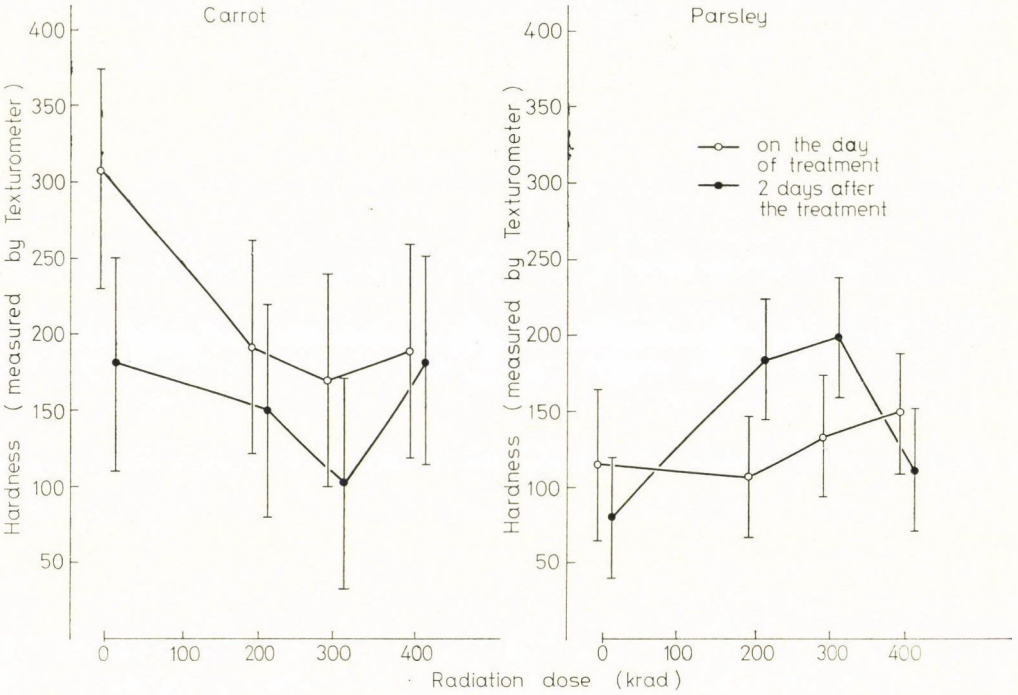


Figure 6. Results of texturometer tests upon samples stored at room temperature (20—25 °C), hardness values and average of their standard deviations (third experiment)

The increase of the cell count is much slower in samples kept in cold store. The control samples, with higher initial cell count, reached the 10^8/g level on the 7th day of storage, while that of the irradiated samples was about 10^7/g on the 14th day. The 10^6/g level was reached by the irradiated samples between the 6th and 8th day.

The most probable count of moulds was below 10^4/g on the day of treatment in all the samples. At room temperature the viable cell count of the samples not treated and treated with 200 krad approximated the maximum mould count (5×10^5) permitted in the Hungarian sanitary directives (ORMAY, 1970). On the 14th day of storage of the samples kept in cold store, this limit was exceeded by the control samples and approximated by the samples treated with 200 krad.

2.3. Instrumental measurement of the consistency of carrots and parsley

The average values and the deviations from the mean of the results obtained in texturometer measurements at each dose level are given in Figs 6 and 7.

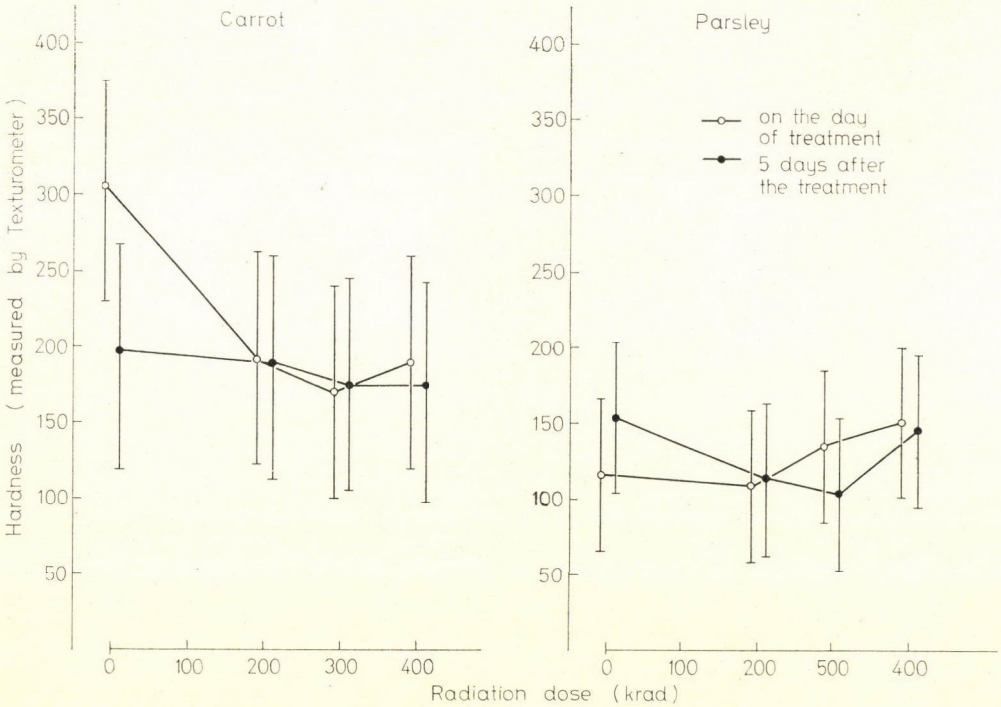


Figure 7. Results of texturometer tests of samples stored in cold store (9—12 °C), hardness values and average of their standard deviations (third experiment)

The results were tested by analysis of variance (SVÁB, 1967) and the effect of radiation dose and storage time upon the consistency of carrots and parsley was studied. The results are given in Tables 3 and 4.

A statistically significant softening was observed in the consistency of irradiated carrots. The differences between dose levels were insignificant.

The difference between the treated and untreated samples became unambiguous on the second day at room temperature and on the 5th day in samples stored at lower temperature.

In the case of parsley (Table 4) the effect neither of irradiation, nor of storage time exceeded that due to inhomogeneity of the samples.

Table 3

Consistency of carrots as affected y dose level

The mean of texturometer values belonging to the irradiated samples related to the mean value of the control or samples receiving different treatment (ΔH) and the t values of difference according to Student

Storage time, days		0—200 krad		0—300 krad		0—400 krad		200—300 krad		200—400 krad		300—400 krad	
		ΔH	t	ΔH	t	ΔH	t	ΔH	t	ΔH	t	ΔH	t
at ambient tem-	0	112.0	2.9**	132.7	5.0***	114.0	4.3***	20.7	0.7	2.0	0.07	−18.7	0.9
perature (20—25°C)	2	29.0	1.2	77.0	2.8**	−4.2	−0.1	48.0	1.9*	−33.2	−1.1	−81.2	−2.7**
in cold store	0	112.0	2.9**	132.7	5.0***	114.0	4.3***	20.7	0.7	2.0	0.07	−18.7	0.9
(9—12°C)	5	4.6	0.1	22.1	0.9	24.8	1.0	17.5	0.6	20.2	0.7	2.7	0.1

Consistency of carrots as affected by storage time

The mean consistency value of the sample as measured during storage related to the initial mean value at the same dose level (ΔH) and t values of the differences according to Student

Dose (krad)	at room temperature (20-25 °C)		in cold store (9—12 °C)	
	0—2 days		0—5 days	
	ΔH	t	ΔH	t
0	123.0	4.1***	106.2	3.3***
200	40.0	1.4	−1.2	−0,03
300	67.3	2.4*	−4.4	−0.2
400	4.8	0.9	17.0	1.05

Level of significance

* = P $\leq$ 5%

** = P $\leq$ 1%

*** = P $\leq$ 0.1%

Table 4

Consistency of parsley as affected by radiation dose

The mean of texturometer values belonging to the irradiated sample related to the mean value of the control or samples receiving different treatment (Δ H) and the t value of difference according to Student

Storage time, days		0–200 krad		0–300 krad		0–400 krad		200–300 krad		200–400 krad		300–400 krad	
		ΔH	t	ΔH	t	ΔH	t	ΔH	t	ΔH	t	ΔH	t
ambient temperature (20–25 °C)	0	8.3	0.3	−18.2	−0.65	−32.5	−1.65	−26.6	−1.0	−40.8	−2.0*	−14.3	−0.7
	2	−108.0	−11.3***	−126.5	−2.7*	−37.5	−1.1	−18.5	−0.4	70.5	2.1*	88.9	1.8
in cold store (9–12 °C)	0	8.3	0.3	−18.2	−0.65	−32.5	−1.65	−26.6	−1.0	−40.8	−2.0*	−14.3	−0.7
	5	65.9	3.4***	76.1	4.8***	57.5	4.3***	10.2	0.5	−8.4	−0.4	−18.6	−1.1

Consistency of parsley as affected by storage time

The mean hardness value of the samples as measured during storage related to the initial mean value at the same dose level (Δ H) and the t value of the difference according to Student

Dose (krad)	at room temperature (20–25 °C)		in cold store (9–12 °C)	
	0–2 days		0–5 days	
	ΔH	t	ΔH	t
0	41.8	1.6	−61.0	−3.3**
200	−74.4	2.7**	−3.4	−0.1
300	−66.4	−1.6	33.4	1.4
400	36.8	−1.5	29.0	2.7*

Level of significance

* = $P \leq 5\%$

** = $P \leq 1\%$

*** = $P \leq 0.1\%$

2.4. Softening of carrots upon cooking

To establish whether the cooking time requirement of carrots is reduced by irradiation carrots of uniform size were used.

A radiation dose of 300 krad caused a statistically significant softening in the consistency of the raw carrot (Table 5).

The average consistency values, belonging to a given cooking time of the treated and untreated carrots were compared in tests. As seen in the table, the difference between the irradiated and untreated samples was significant only in the first 10—15 minutes, after that became irrelevant.

In order to analyse the extent and trend of change the consistency values belonging to given cooking times were plotted in a log-log system of coordinates, except the values of raw carrots (Fig. 8). The regression curves belonging to the points were plotted on the basis of regression analysis. The slopes of the curves were compared. The statistical test of the difference between the two calculated regression coefficients resulted in: $t = 3{,}8461^{**}$, meaning that the two curves differ significantly at an error level of 1% probability.

Table 5

Consistency change with time of

(Mean values of hardness as measured with Texturo-

		Cooking			
		0	5	10	15
Control	mean value	568.2	410.9	178.0	105.3
	standard deviations	32.7	38.3	49.2	20.9
300 krad	mean value	514.1	154.7	125.7	109.4
	standard deviations	35.4	26.1	34.9	37.8
t test		4.3***	21.26***	3.31***	−0.37

Level of significance
* = P ≤ 5%
** = P ≤ 1%
*** = P ≤ 0,1%

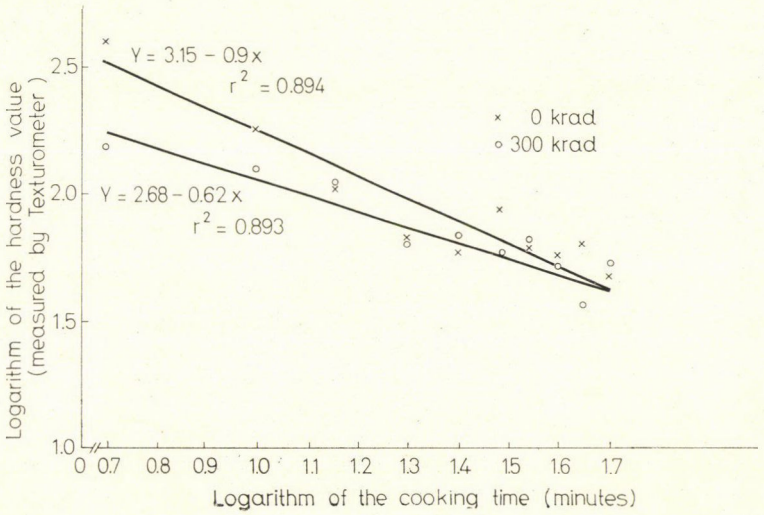


Figure 8. Regression lines of the changes in the consistency of carrots as caused by ionizing radiation and cooking, respectively, and their analysis

irradiated carrots during cooking

meter, standard deviations and Student's t value)

time (minute)						
20	25	30	35	40	45	50
66.4	58.6	86.1	60.6	56.3	62.6	47.4
8.6	20.5	35.2	27.8	9.4	10.4	14.4
63.7	67.8	57.4	64.7	51.0	36.0	52.4
23.2	17.9	19.7	11.9	9.2	8.4	13.3
0.42	−1.30	2.72*	−0.52	1.56	7.69***	−0.99

2.5. Sensory evaluation

The panel of judges evaluated the untreated sample, and those treated with 300 and 400 krad, respectively, and stored at room temperature, on the second day, and those kept in cold store on the 3rd and 7th day of storage, by taking a sniff at the pouches. The result of the evaluation is given in Figs 9 and 10.

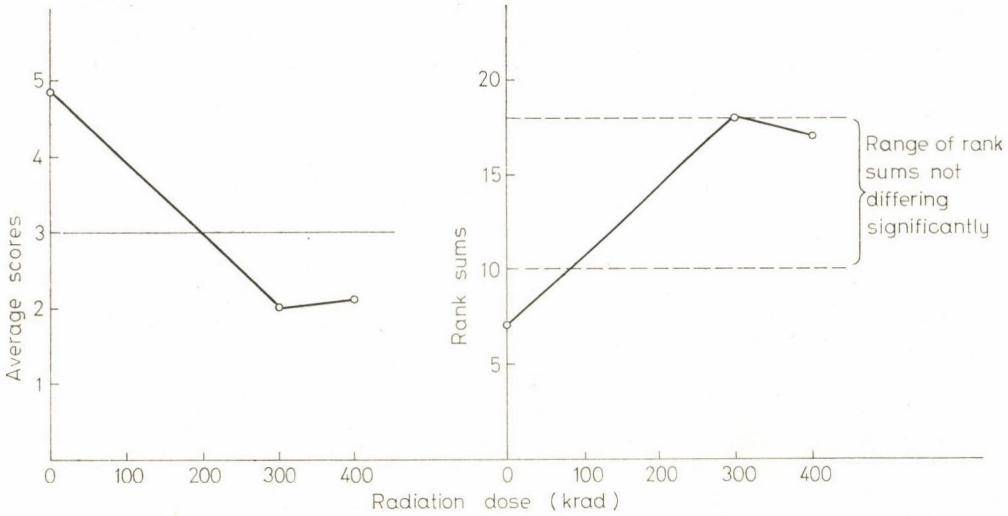


Figure 9. Sensory evaluation of the smell of raw vegetables stored at room temperature (20—25 °C) on the 2nd day of storage. The panel consisted of seven members. The significance test was carried out at P = 5% level. The dashed lines enclose the rank sums not differing significantly. The horizontal line drawn to score 3 in the graph of average scores marks "just fair" sensory level. At each dose level the samples were tested as a whole and not the individual vegetables (third experiment)

The smell of the untreated samples was that of fresh vegetables, whereas that of the irradiated samples was sweetish and differed from that of the fresh vegetable. This difference is shown in the results given in the tables. However, the sweetish off-odour of the samples disappeared after the pouches had been aerated. The initially significant difference in the smell disappeared by the 7th day of storage (Fig. 10).

In another evaluation the taste of samples cooked for 15 minutes under 0,5 kpond/cm^2 gauge pressure was tested on the 3rd day of storage. The vegetables of different varieties were tested separately. The average values of the scores as well as the rank sums are given in Fig. 11.

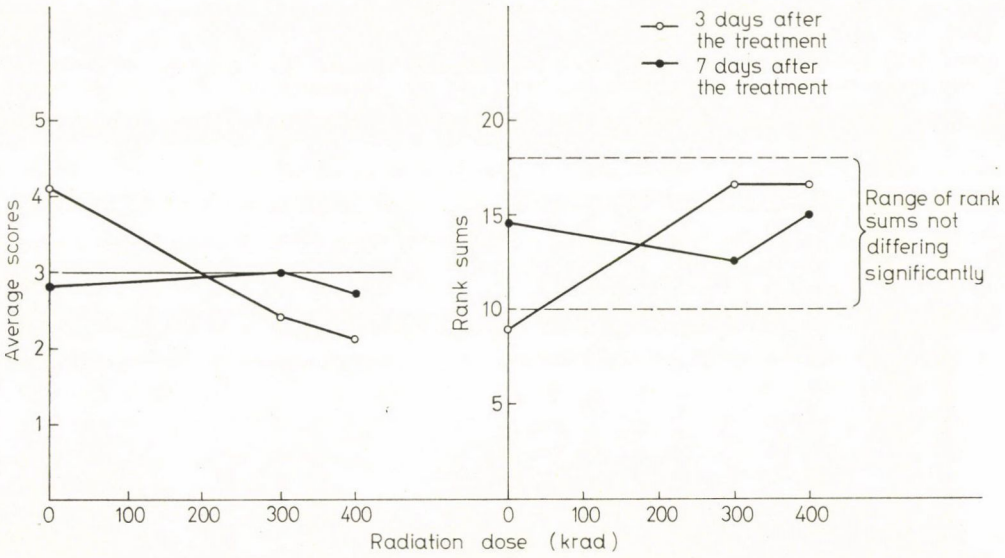


Figure 10. Sensory evaluation of the smell of raw vegetable samples stored at 9—12 °C temperature, on the 3rd and 7th day of storage. The panel consisted of seven members. The significance test was carried out at P = 5% level. The dashed lines enclose the rank sums not differing significantly. The horizontal line drawn to score 3 in the graph of average scores marks the sensory level "still fair". At each dose level the samples were tested as a whole and not the individual vegetables (third experiment)

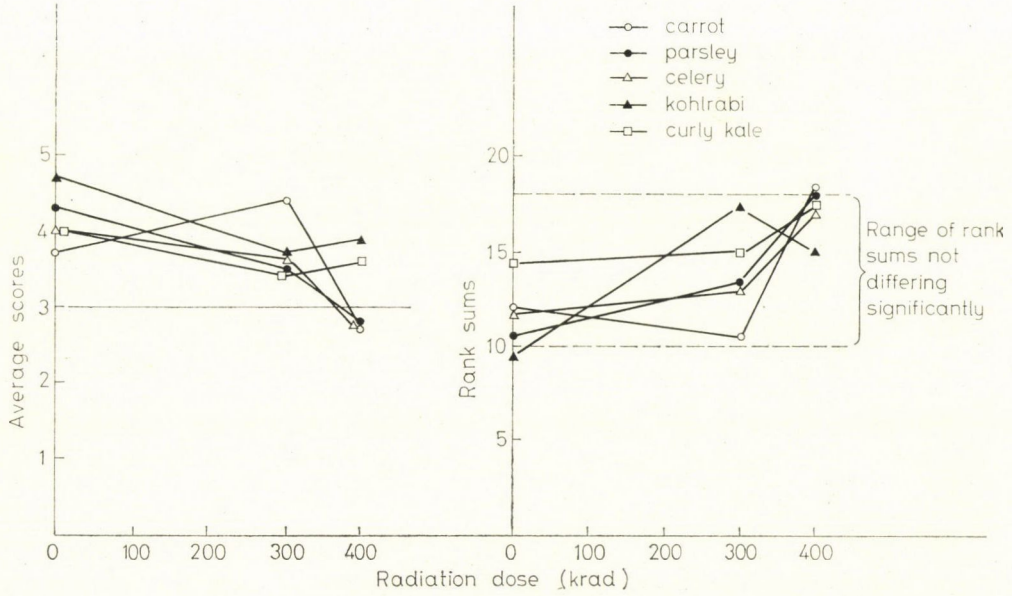


Figure 11. Sensory evaluation of the taste of cooked samples stored at 9—12 °C on the third day after irradiation. The panel consisted of 7 members. The significance test was carried out at P=5% level. The dashed lines enclose the rank sums not differing significantly. The horizontal line drawn to score 3 in the graph of average scores marks the sensory level "still fair" (third experiment)

3. Conclusions

It was shown in these experiments that the spoilage of prepared mixed vegetables, as established by sensory evaluation could be delayed by 2 days with radiation treatment at 200—300 krad dose level and storage either at room temperature or in cold store (Tables 1, 2, Figs 1, 2). The dose range of 200—300 krad reduced the viable cell count of the mixed vegetables by about two orders of magnitude. The difference observed in the initial cell count of the treated and untreated samples remained constant during storage in spite of increasing cell count till the appearance of spoilage as established by sensory evaluation (Figs 3, 4, 5).

Of the vegetables investigated parsley and celery were most liable to spoilage. Carrots had a much better keeping quality. In kohlrabi the darkening is the factor limiting storage, however this is independent of radiation treatment. The highest resistance was shown by curly kale, even after two weeks of cold storage it was sound and of fresh character in the untreated samples as well.

It was established by instrumental measurement of the consistency that a treatment of 200 krad was sufficient to cause a statistically highly significant softening ($P = 1\%$).

In the case of parsley even radiation doses of 300—400 krad did not effect measurable softening in comparison to the untreated samples. Since untreated carrots softened rapidly during storage, while the irradiated ones showed practically no change, the initial difference in consistency between the carrot samples became equalized during storage (Figs 6, 7 and Tables 3, 4). The softening of carrots was investigated by several authors (GLEGG *et al.*, 1956; BOYLE *et al.*, 1957). In these experiments the threshold dose causing softening was found to be between 23 and 178 krad, and this is in good accord with our findings.

On investigating the softening of carrots during cooking it was established that the radiation treated samples softened much more rapidly in the first five minutes of cooking than the untreated ones. However, during further cooking the softening rate of the untreated carrots was higher than of those irradiated with 300 krad (Fig. 8) and thus in the 5th minute of cooking the difference in the consistency of the treated and untreated samples disappeared (Table 5). These observations support those found by various authors as regards the good cooking capacity of radiation treated dried vegetables (SCHROEDER, 1962; MARKAKIS *et al.*, 1965; STADEN, 1966; METLITSKIY *et al.*, 1967; UMEDA *et al.*, 1969; PAUL *et al.*, 1969). Similar observations were made in the laboratory of this Institute (KÁLMÁN & FARKAS, 1970). The sensory tests permit of the conclusion that because of the appearance of off-odour and off-flavour it is not expedient to apply doses above 200—300 krad.

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TRANSPORTATION AND STORAGE STUDIES ON IRRADIATED ALPHONSO MANGOES

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Control as well as skin coated (6% "Myvacet") and/or irradiated (25 to 35 krad) mangoes were packed in baskets in dry paddy straw and shipped by rail over a distance of 1 100 kilometers from Bombay. After storage there at a temperature of 25 to 32 °C for a period of 8 days, the baskets where shipped back. After receipt in the laboratory, the fruits were kept under observation for 5 more days and then scored for ripening and marketability as assessed by appearance, skin gloss and typical flavour. 100 per cent of fruits skin-coated and irradiated were marketable at the end of the experiment. None of the unirradiated control fruits were saleable by this time, while 66% of the irradiated fruits were in acceptable condition.

In further experiments one dozen mangoes each from control, irradiated and skin-coated and irradiated groups were wrapped individually in tissue paper and packed tightly in a plywood box. This consignment was air-lifted from Bombay to Budapest (a distance of about 5 600 kilometers). The time interval between irradiation and their receipt in Hungary was 4 days. The mangoes were assessed for their storage characteristics by various parameters that included physiological loss in weight, puncture resistance of the skin, pulp texture, carotenoids, acidity and organoleptic attributes, at various periods during storage. It was found that ripening and senescence are strikingly delayed in mangoes by skin-coating and radiation treatment. The treated mangoes retained their skin-resistance while the disappearance of chlorophyll and formation of carotenoids were substantially delayed. Weight loss in storage was lower and reduction of acidity and formation of sugars in the fruit flesh took place later. Organoleptically, the fruits subjected to the combination treatment were good, followed by the irradiated fruits. These studies point to the feasibility of the combination treatment in prolonging the shelf life of fruits significantly to enable their transport to distant countries.

Post-harvest handling of fresh fruits subjects them to a great variety of damaging vibrations and impacts. Vibrations in transit may reflect irregularities in road bed or wheels, transmitted to the load while impact bruises may occur at any time during and after harvest if the fruit drops more than a few inches. SOMMER et al. (1960) found that the vibrational injuries were much less severe when the fruits were cooled, which is in turn due to the fact that fruits would be turgid if cold and flaccid if warm. The puncture test on Tilton apricots averaged, according to the same authors, 10 lbs when cold and 6.7 when warm. This, therefore, points to the desirability of keeping fruits in hard condition during transit, since vibrational injuries lead to the rupture of skin epidermal cells and the consequent surface dehydration, wrinkling, fungal and mold attack and also hastens ripening and senescence.

Earlier work on delaying ripening in mangoes by irradiation show the feasibility of the process (DHARKAR *et al.* 1966a, b; DHARKAR & SREENIVASAN, 1966; SOLANAS & DARDER, 1966; MUMTAZ *et al.* 1968; KOVÁCS *et al.* 1969). On the other hand, radiation damage may be amplified considerably during transit by rail or road, over long distances and the maximum radiation dose (about 200 krad) tolerable for tissue softening would have to be brought down drastically if irradiated fruits have to be transported (MAXIE & ABDEL-KADER, 1966). From all these considerations, it was desirable to compare the behavior of irradiated and control mangoes during transit, under the hot and humid conditions prevalent in India and also while being air lifted to other countries.

1. Materials and methods

Mature unripe Alphonso mangoes which were hard and having olive green skin color were selected for these studies through a local shipping agent.

1.1. Rail transit in India

Two dozen fruits for each of the treatments viz., unirradiated control, irradiated (25 to 35 krad), skin-coated by 6% emulsion of "Myvacet", an acetylated monoglyceride (Distillation Products Industries, Rochester, N.Y., USA) and then irradiated (25 to 35 krad) were packed tightly in separate baskets in dry paddy straw as is the usual commercial practice in India and shipped unrefrigerated by rail to Bangalore, a distance of about 1 100 kilometers from Bombay. After storage at Bangalore at ambient temperature (25° to 32 °C) for a period of eight days, the baskets were shipped back to Bombay, the rail journey each way taking about 36 hours. Temperatures during transit were quite high, going up to 42 °C. After receipt in the laboratory the fruits were kept under observation for five more days and then scored for ripening and marketability as assessed by appearance, skin colour and typical flavour.

Organoleptic evaluation of the ripe fruits was carried out by a panel of six judges, who scored on a 9 point hedonic scale.

1.2. Air-lifting of mangoes to Hungary

Control, irradiated and skin-coated and then irradiated fruits were packaged in a plywood (0.5 thick) box, partitioned to separate the variously treated samples. The fruits were wrapped individually in tissue paper and then packed tightly with the aid of paper cuttings. Ventilation holes on the plywood box were covered from inside with 20 mesh wire gauze to prevent insect infestation. The consignement was air lifted to Hungary and reached the

Central Food Research Institute, Budapest, a distance of about 5 600 kilometers from Bombay within four days on 11 June 1969.

The consignement consisted of 10 control, 9 irradiated and 10 skin-coated and then irradiated mango samples. The fruits were stored at 20—22 °C and 50% relative humidity.

Each fruit in every batch was weighed every day to follow weight loss. From time to time colour photographs were taken to record ripening changes and two fruits from each batch were used to determine texture, organoleptic qualities and also carotenoids, sugars, titratable acidity and pH. In order to differentiate between the individual fruit samples, except for the carotenoid determination, the two parallel samples were tested separately. Carotenoid content was determined from the combined pulp of the two fruits.

The loss in weight was established by comparing the weight of each fruit with its initial weight as taken at the beginning of storage in Budapest.

Instrumental texture determination was carried out after 2, 5 and 9 days of storage (on 13, 16 and 20 June 1969), by the automated "Labor 5264" type penetrometer which measures the puncture resistance of the fruit. This instrument records the depth of penetration of the standard cone (total length of cone, 44.6 mm; angle 30° and height 15.2 mm) at the standard load of 150 $\pm$ $\pm$ 0.05 g at 25 °C, during 5 seconds, perpendicular to the surface of the sample. One degree on the penetrometer scale is equivalent to 0.1 mm depth of penetration. Five determinations were made to measure the resistance of the skin and the consistency of the fruit flesh, respectively, by use of fruits with and without skin.

The fruit flesh of the mangoes was organoleptically tested by ranking. The sensory evaluations were carried out after 5 and 9 days of storage (on 16 and 20 June 1969), with the fruit that was used for the instrumental measurement of texture. The parts of the fruit damaged by the penetrations were used to determine the sugar and acid content.

The judges evaluated colour, aroma, flavour and consistency of two fruits of each batch (i.e. 6 fruits altogether) according to the 9 point hedonic scale. Due to unavailability of mangoes on the Hungarian markets, the judges had no experience with testing of this fruit and since their judgement would not, therefore, reflect the actual quality of the fruit, it was decided to score on the basis of the comparatively unambiguous characteristics as follows:

colour	maximum score 9: bright orange coloured fruit flesh
	minimum score 1: pale yellowish green, lemon like fruit flesh
aroma	maximum score 9: the most intense, aromatic smell
	minimum score 1: the most feeble smell
taste	maximum score 9: the sweetest fruit
	minimum score 1: the most tart fruit

consistency maximum score 9: the softest, butterlike consistency
minimum score 1: the hardest stringy consistency

The scores were ranked according to KRAMER's (1960) method and the rank sum was computed for each fruit.

Titratable acids: 10 g fruit flesh were pulped by attrition, suspended in water, diluted to 100 ml and strained, 20 ml of the filtrate were titrated with 0.1 N NaOH, using phenolphtalein indicator.

pH: pH determination was carried out in twofold dilution of the fruit pulp.

Carotenoids: 5 g of the pulp of each fruit used for sensory and chemical tests was mixed, in the presence of anhydrous Na_2SO_4, with 40 ml acetone in a "Biomix" homogenizer, for 5 minutes. The extract thus obtained was filtered through a glass funnel and the clear filtrate diluted to 10 ml with acetone. The stock solution was then measured at 445 nm in a PERKIN—ELMER, No. 137 UV spectrophotometer.

2. Results

2.1. Rail shipment from Bombay to Bangalore and storage in Bangalore

Data presented in Table 1 show the combination treatment of skin-coating and irradiation to be best, with 100% of these fruits being marketable (as assessed by appearance, skin color and typical flavour) at the end of the

Table 1

Transportation studies on irradiated mangoes
(Temp. 26°—42 °C)

Storage time, days	Control		Irradiated		Skin-coated and irradiated	
	Ripe fruit %	Marketable %	Ripe fruit %	Marketable %	Ripe fruit %	Marketable %
3	10	100	—	100	—	100
6	100	90	20	100	—	100
9		50	50	100	10	100
12		10	100	100	60	100
15		0		66	100	100

Two dozen each from control, irradiated and skin-coated and then irradiated Alphonso mangoes were transported from Bombay to Bangalore by rail, a distance of about 1 100 kilometers. After storage at Bangalore for eight days at room temperature, all batches were returned to Bombay by rail. Transportation both ways and storage were unrefrigerated.

experiment while none of the unirradiated control fruits were saleable. The fruits which were subjected to irradiation alone showed 66 % marketable fruits.

Table 2 shows the texture of transported mangoes as measured with the Magness—Taylor device. The averages of the figures show that the skin-coated and irradiated mangoes were in optimum condition, whereas the control mangoes have softened appreciably. The "irradiated only" group is also in a turgid condition and showing penetration figures nearer to the skin-coated and irradiated group.

Table 3 shows the averages of the organoleptic tests carried out after receipt of the fruits at Trombay. Highest ratings were scored for both the "skin-coated irradiated" and the "only irradiated" fruits, whereas the controls were totally unacceptable.

A photograph of typical samples of shipped mangoes from each of the groups at the end of the experimental period of 15 days (Fig. 1) shows that while the unirradiated controls (marked 1) are overripe, blemished and unsaleable, the irradiated ones (marked 2), have a better appearance and the skin-coated and irradiated fruits (marked 3) are best and are still in fresh-like condition.

Table 2

Texture of the transported Alphonso mangoes determined by a Magness—Taylor penetration device at the end of the experiment (15th day post-irradiation) (Bombay—Bangalore—Bombay)

Treatment	Pressure required to penetrate fruit with skin	Average ± standard deviation
Control 1	8; 8.5; 9; 8; 8.5.	8.8±0.6 a*
Control 2	9.5; 9; 8.5; 9.9; 9.	
Irradiated 1	13; 13.5; 13.5; 13; 13.	13.6±0.6 b
Irradiated 2	14.5; 14.5; 13.5; 14; 14.0.	
Skin-coated and irradiated 1	13.5; 16.0; 16,0; 15.0; 16.0.	15.7±0.9 c
Skin-coated and irradiated 2	16.5; 16.5; 16,0; 15.5; 16.0.	

* Means analyzed by analysis of variance and the Duncan multiple range test. Means not followed by the same letter are significantly different at the $\alpha \leq 0.05$ level.

Table 3

Acceptability of the transported Alphonso mangoes determined by a panel of six judges, at the end of the experiment (15th day post-irradiation) (Bombay—Bangalore—Bombay)

Treatment	Organoleptic ratings on nine point hedonic scale	Average ± standard deviation
Control 1	2; 3; 2; 1; 2; 3.	2.5±0.9 a*
Control 2	3; 3; 2; 4; 2; 3.	
Irradiated 1	6; 5; 6; 5; 7; 6.	5.9±0.8 b
Irradiated 2	6; 7; 7; 6; 5; 5.	
Skin-coated and irradiated 1	7; 7; 8; 7; 8; 6.	7.0±0.7 c
Skin-coated and irradiated 2	6; 7; 7; 8; 6; 7.	

* Means analyzed by analysis of variance and the Duncan multiple range test. Means not followed by the same letter are significantly different at the $\alpha \leq 0.05$ level.

2.2. Air-lifting from Bombay to Hungary and storage in Budapest

The condition of the fruits on the day of receipt (on 11 June 1969) was as follows:

"control" batch: from the point of view of ripeness inhomogeneous with green and yellowish fruits

"irradiated" batch: contained fruits more homogeneous as regards skin colour, mostly yellowish-green. None of the individual fruits was as green as the greenest fruits in the control batch, but none was as yellow as the yellow fruits in the control.

"skin-coated irradiated" batch: uniformly dark green

The change in colour of the skin during storage at 20—22 °C is shown in the colour pictures taken on the day of receipt and after 2 and 5 days of storage, respectively (Fig. 2). The colour of the flesh of fruits as observed after 5 and 9 days of storage is shown in Fig. 3 and Fig. 4.

Loss in weight: Weight losses during storage are shown in Fig. 5. It is apparent from the results that there was a linear correlation between weight

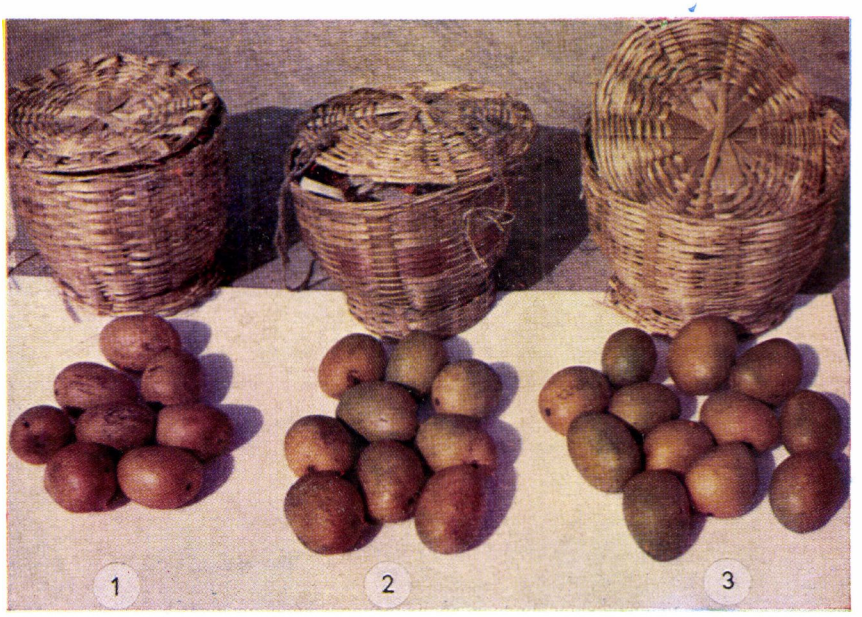

Figure 1. The condition of the typical samples of mangoes at the end of the experimental period of transportation and storage in India

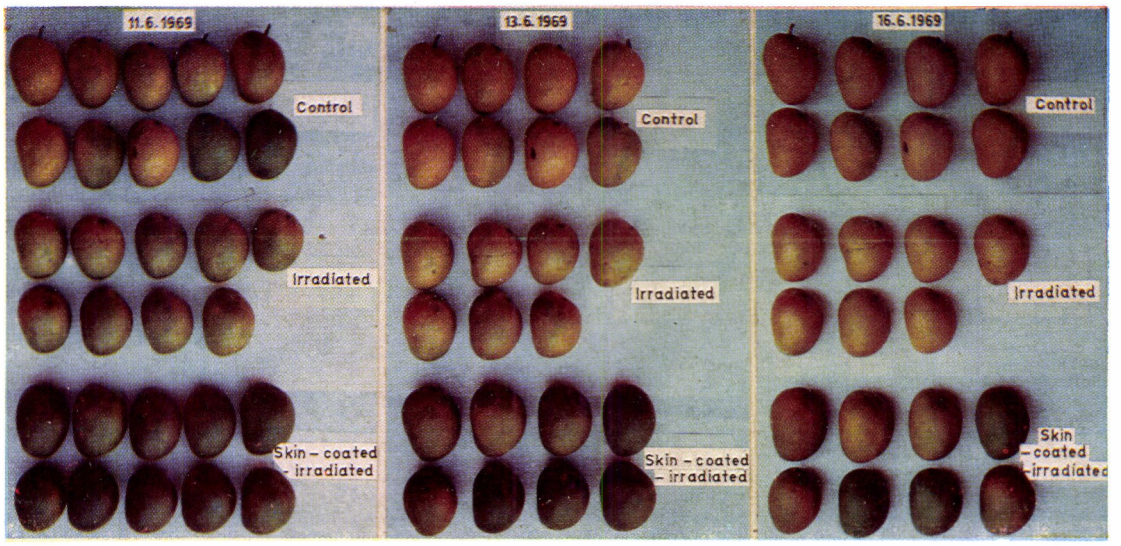

Figure 2. The change in colour of the samples of mangoes during storage. (Air-lifting from Bombay and storage in Budapest).

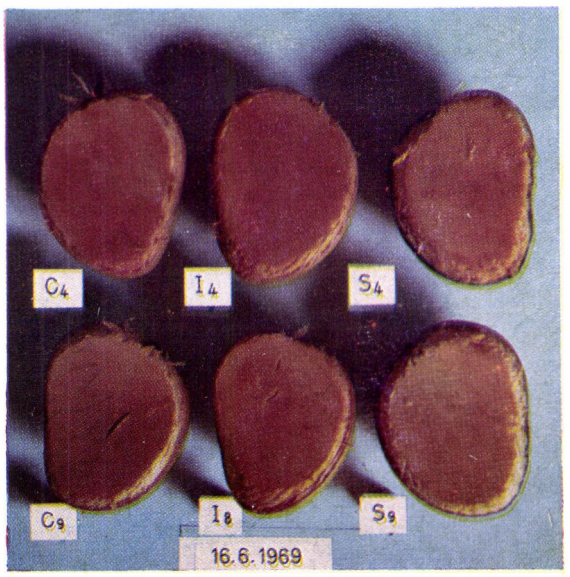

Figure 3. The colour of flesh of fruits after 5 days of storage. C_4, C_9: control; I_4 I_8: irradiated; S_4, S_9: skin-coated and irradiated

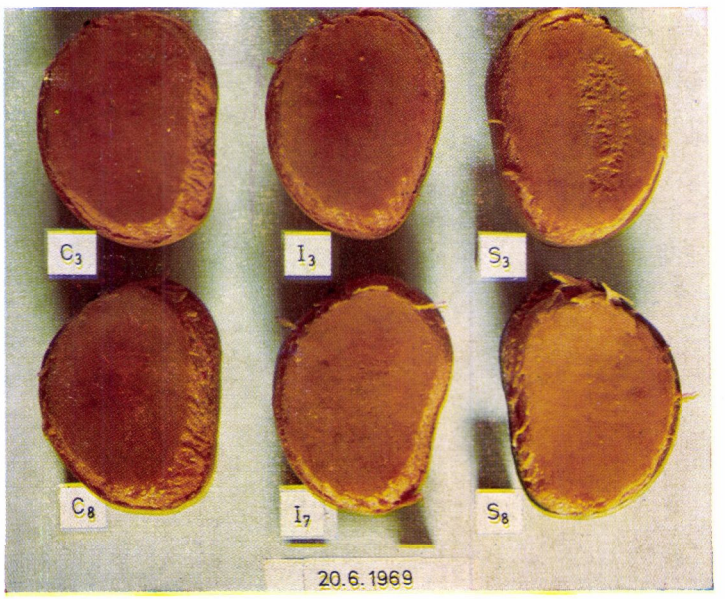

Figure 4. The colour of flesh of fruits after 9 days of storage. C_3, C_8: control; I_3, I_7: irradiated; S_3, S_8: skin-coated and irradiated

loss and time and the loss in the irradiated samples and even more so, that in the skin-coated (irradiated) samples was significantly lower than that in the control. The slope of the regression curves of the control and the irradiated sample differed at $\alpha \leq 0.05$ level ($t = 2.41$; $f = 22$), and the slope of the regression curves of the control and the skin-coated and irradiated sample at $\alpha \leq 0.001$ level ($t = 4.38$; $f = 28$).

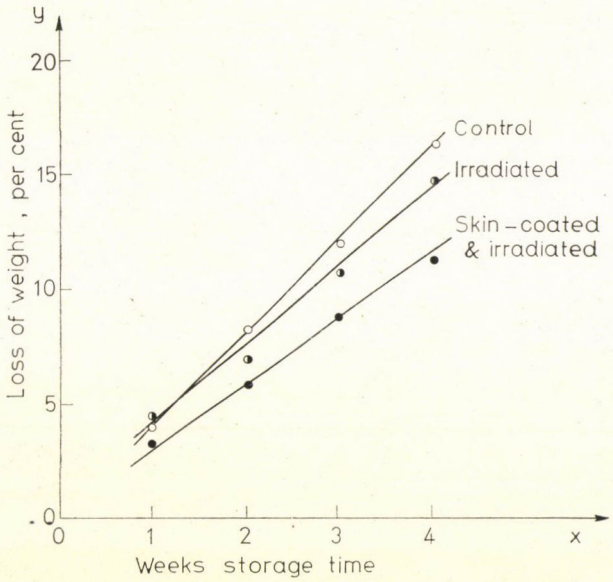

Figure 5. Loss of weight in the mangoes during storage. The graph shows the average values of weight losses and the regression curves. Characteristics of the regression curves: control: $y = 4.07x - 0.08$, $r = 0.9896$*, irradiated: $y = 3.37x + 0.78$, $r = 0.9752$*, skin-coated and irradiated: $y = 3.02x + 0.005$, $r = 0.9758$*
*r significantly differs from 0 at $\alpha \leq 0.001$

The penetrometer measurements during storage are summarized in Table 4. As seen, the skin resistance of the skin-coated and irradiated samples was significantly higher than that of the untreated or only irradiated samples during the first stage of storage. The softening of the thick, leathery skin of the skin-coated and irradiated samples lagged behind that of the control samples with at least 2—3 days during storage. On the other hand the texture of flesh of the radiation treated as well as the skin-coated and irradiated samples was softer to a significant extent than that of the control samples. However, the differences were compensated during storage.

The differences as established by instrumental and chemical analysis between the untreated and the skin-coated and irradiated samples were confirmed by the results of the sensory evaluations carried out on the 5th and 9th day of storage (Table 5). The titratable acid content, the total and reducing

Table 4

Texture of mangoes as established by penetrometer (Bombay — Budapest)

Treatment	Penetrometer values (average ± standard deviation)					
	Through the skin			Peeled		
	2*	5	9	2	5	9
Control	20.7±9.4 bc	35.1±5.5 de	49.5±5.2 h	115.7±19.9 i	132.2±14.5 j	159.5±10.4 l
Irradiated	25.1±4.0 c	33.0±6.1 d	39.7±3.7 f	138.4± 9.6 j	150.4± 9.4 k	140.4±10.6 jk
Skin-coated and irradiated	15.5±6.5 ab	26.7±4.4 c	42.4±6.6 fg	131.2±16.3 j	154.7± 9.7 k	158.7±13.6 l

Means analyzed by analysis of variance and the Duncan multiple range test. Means in any row not followed by the same letter are significantly different at the $\alpha \leq 0.05$ level.

* Days of storage at room temperature.

Table 5

Results of organoleptic test

Treatment	Sample	Colour of flesh		Odour		Taste		Texture	
		mean score	rank sum	mean score	rank sum	mean score	rank sum	mean score	rank sum
	After 5 days of storage*								
Control	C_4	6.9	38.5	7.3	28.5	7.1	32.5	6.4	37.5
	C_9	6.5	41.5	6.2	42.0	6.4	41.5	6.8	37.0
Irradiated	I_4	8.9	16.0	8.0	25.5	7.9	26.0	7.5	32.5
	I_8	8.6	17.0	7.1	29.5	6.9	37.5	7.1	32.5
Skin-coated and irradiated	S_4	5.3	52.5	4.3	57.0	5.6	44.0	5.4	47.5
	S_9	3.8	65.5	5.5	48.5	5.3	49.5	6.4	41.9
	After 9 days of storage**								
Control	C_3	7.9	27.5	8.7	15.5	7.8	24.5	8.0	31.5
	C_8	8.8	16.0	8.1	20.0	8.4	18.5	6.6	37.0
Irradiated	I_3	7.6	31.5	6.9	38.0	5.2	48.5	6.8	32.5
	I_7	7.2	30.5	6.3	43.0	6.7	35.0	6.1	41.5
Skin-coated and irradiated	S_3	6.2	45.5	6.9	39.0	6.8	35.5	7.1	34.0
	S_8	4.6	59.0	6.1	54.5	5.3	48.0	7.2	33.5

* Number of panelists: 11 — Lowest significant rank sum: 25 — Highest significant rank sum: 52

** Number of panelists: 10 — Lowest significant rank sum: 22 — Highest significant rank sum: 48

——— values significantly lower than rank sums contained in the same column ($\alpha \leq 0.05$)

═══ values significantly higher than rank sums contained in the same column $\alpha \leq 0.05$)

Table 6

Chemical characteristics of mangoes

Storage time days	Treatment	Titratable acids**	Total sugar in fruit flesh, %	Reducing sugar in fruit flesh, %	Optical density of carotenoid-extract at 445 nm, E_{1cm}
5	Control	2.4±0.1* a	7.0±0.7 c	1.6±1.0 d	0.90±0.10 ef
	Irradiated	2.3±0.2 a	7.4±0.3 c	1.4±0.1 d	1.17±0.04 e
	Skin-coated and irradiated	9.8±1.3 b	5.3±2.3 c	0.4±0.1 d	0.62±0.05 f
9	Control	1.9±0.1 a	8.9±0.9 c	1.2±0.2 d	0.95±0.14 e
	Irradiated	2.1±0.1 a	7.5±0.1 c	1.0±0.1 d	1.09±0.06 e
	Skin-coated and irradiated	2.5±0.1 a	8.9±0.6 c	2.0±1.0 d	0.60±0.04 f

* Means of replicates ± standard deviations. Means analyzed by analysis of variance and the Duncan multiple range test. Means within a column not followed by the same letter are significantly different at the $\alpha \leq 0.05$ level.

** ml-s of 0.1 N NaOH for 10 g fruit flesh.

sugar content and the results of carotenoid measurements are shown in Table 6. In accordance with the difference in the titratable acid content detectable on the 5th day of storage a significant difference was observed in the pH of the flesh of the untreated and skin-coated and irradiated fruits (pH = 4.9—5.5 as against pH = 3.8).

3. Conclusions

The data obtained in the experiments both in India and Hungary show that ripening and senescence are significantly delayed in mangoes by a combined treatment of skin-coating and irradiation, even after transportation to a distant country. As a combined effect of skin-coating and radiation treatment the skin of the mangoes retains its resistance and disappearance of the chlorophyll providing the green colour of the skin, the reduction of the acidity of fruit-flesh and the formation of the orange red carotenoids are substantially delayed. The loss of weight during storage is also lower. Though initially the consistency of the flesh of the irradiated fruits is softer than that of the untreated ones, the difference becomes compensated within a few days by the slower after-ripening of the radiation treated samples. At the end of 15 to 20 days of transport and storage, due to the delayed ripening, the skin-coated and irradiated fruits are the most palatable. Thus these studies indicate this combined treatment to be a feasible proposition for the protection of mangoes even during unrefrigerated transport.

*

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INVESTIGATION OF THE SUITABILITY OF GREEN PEA VARIETIES FOR QUICK-FREEZING

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The series of experiments on the suitability of the more important vegetable varieties for quick-freezing and on their keeping time at various temperatures included tests on five green pea varieties.

The material to be quick-frozen was blanched at 95 °C for 2 minutes, then packed into cardboard boxes each containing 0.4 kg. The material then was quick-frozen at −30 °C. After quick-freezing the boxes were stored at −30, −20 and −10 °C.

From the investigation of the raw materials a good correlation was established between the texture measured by means of the Finometer as expressed in Tenderometer degrees (Table 1) and the alcohol insoluble solids (Table 2), and both were characteristic of the quality of the raw material. The solids content is proportional to the alcohol insoluble solids (Table 3). The colour of the green pea varieties was measured immediately after picking by spectrophotometry of the acetone extract (Table 4). The Mignon variety has the darkest colour, followed in order by the Kelvedon, Grüne Perle, Rhine dwarf and finally by the Vitalis variety. Since the refrigeration industry prefers the darker green pea varieties, from the aspect of colour the first three are more suitable for quick-freezing.

Changes in the texture of green pea varieties caused by various technological operations (blanching, cooking, quick-freezing, storing at various temperatures, thawing after storage at low temperature followed by cooking) compared to untreated samples were recorded by the Finometer and the results expressed in Tenderometer degrees (Figs. 1 and 2).

We found that in each variety, compared to raw peas, blanching caused a drop of about 50% in consistency. The reduction in consistency after cooking at 100 °C for 9 minutes was almost the same as that obtained by blanching. Comparison of the texture of quick-frozen and stored green peas to the blanched peas prior to freezing shows a hardening of the texture during storage. The maximum increase in hardness in samples stored at −30 °C for 12 months was 35%, in those stored at −20 °C for the same period 66%. Investigation of the same samples after cooking at 100 °C for 5 minutes indicated, on the other hand, a sudden drop in the texture values. If compared to samples cooked after picking at 100 °C for 9 minutes the change in the texture of initially tender varieties (Kelvedon, Vitalis) may reach a 99% softening, while in the varieties having a harder texture on picking the softening amounted to about 40%.

The results of sensory colour estimation (Figs 3 to 7) show a 30% deterioration in colour during the 4th month when stored at −10 °C. Stored at −20 °C the Vitalis, Mignon and Rhine dwarf varieties suffered measurable changes in colour, while at −30 °C there was practically no change in colour of any of the varieties.

The results of organoleptic taste tests were evaluated for each variety and each storing temperature by mathematical statistical analysis (Figs 8 to 10, Tables 5 to 9). After 4 to 5 months the samples stored at −10 °C gave significantly poorer results than those stored at −20 °C and −30 °C. According to the results obtained with samples stored at −20 °C, with the exception of Mignon, all varieties are suitable for storing up to 12 months. Of the samples stored at −30 °C Mignon alone suffered a 30% deterioration of taste within one year.

Deterioration rate was calculated from the results of taste testing as a function of storage temperature. Figure 11 shows the time needed for a 30% deterioration in taste, while from Fig. 12 the time needed for reaching a 50% deterioration in taste, which represents the limit of palatibility, has been plotted for various storage temperatures.

Summing up the results it appears that from the aspect of quick-freezing the order of the tested varieties is as follows: Kelvedon, Grüne Perle, Vitalis, followed by Rhine dwarf and Mignon. From the aspect of storage for over a year −20 °C is adequate if judged by the results of taste testing, but changes in colour occur at this temperature earlier than at −30 °C.

Though looking back at a past of several years, research related to the preserving of green peas is far from being completed. Experiments have been carried out in many fields to select and process the raw material and to test its quality. Evaluation of the results indicate, however, that these experiments should be continued. Some problems of the varieties have been solved, but the comparative testing of specific properties — from picking to the utilization of the preserved and stored material — is from several aspects still unexplored.

The soil and climatic requirements of green peas (JÁNOSSY, 1955; GRABNER, 1956; SOMOS, 1956; TELEGDY KOVÁTS & HOLLÓ, 1957), their overall heat requirement (VAS & SÁRAY, 1963; CSATÁRI-SZÜCS & KOMJÁTI, 1964; VAS *et al.*, 1965), the effect of texture density on cultivation technical factors (NAGY, 1965), the necessity for the individual cultivation of the various green pea varieties (SCHEFFER, 1962; GALAMBOS, 1963; KILB & JAKAB, 1964; GALAMBOS, 1967; SÁRAY *et al.*, 1967; TÖRÖK, 1967), problems of the suitability for quick-freezing (ALMÁSI & PERÉDI, 1949; ALMÁSI, 1957; TRESSLER & EVERS, 1957; SCHEFFER, 1962; POSGAY & RUTTKAY, 1967; BEKE, 1970), biochemical changes due to storage temperature (VAN ARSDEL *et al.*, 1959; BRODY *et al.*, 1960; JUL, 1961) are discussed in a great number of communications.

With respect to the quality of raw green peas considerable results have been achieved in the elaboration of test methods, including size grading (TÖRÖK, 1967; FAO, 1967a; GUTSCHMIDT, 1968), and specific gravity determination (VAS *et al.*, 1964; SCHALLER, 1965; KÖRMENDY, 1967; SÁRAY *et al.*, 1967; GUTSCHMIDT, 1968), many instruments operating on mechanical principles have been constructed for the measurement of the mechanical properties of green peas, including shear and tensile stength. GRÜNEWALD and KLOTZ (1962), among others, have described various types. The Tenderometer has been in use in the USA since 1937, the Maturometer was suggested by LINCH and MITCHELL in 1950, the Penetrometer by VARENZOFF, the Texturometer was put on the market by the firm M. F. CHRISTEL in the USA in 1938. We use in Hungary the Finometer constructed by VÁGNER and CSENDES in 1968.

Chemical tests include the determination of the alcohol insoluble solids (AIS), investigation of the correlation between the AIS values and the texture as measured by one of the instruments (KEVEI, 1950; A.O.A.C., 1960;

VAS, 1962; KÖLLŐ, 1964; VAS *et al.*, 1964; SÁRAY *et al.*, 1967; FAO, 1967a; SZÁNTÓ, 1968, 1969, 1970; KOVÁCS, 1970a, 1970b).

The experiments published herein, based on the literature and our own experience, were aimed at the ranking and the study of the time-temperature-tolerance of frozen green pea varieties.

1. Materials and methods

1.1. Raw material

For the experiments 5 green pea varieties were obtained from the Herceghalom State Farm. These were in the order of the date of picking:

Mignon	June 11 1969
Rhine dwarf	June 18 1969
Kelvedon	June 20 1969
Grüne Perle	June 26 1969
Vitalis	July 1 1969

Of these varieties the first four have already been used for quick-freezing but the Dutch Vitalis is a variety still under study.

The picked green peas were machine shelled on the Farm, then delivered in paper bags by car to the Institute. The tests started immediately after receipt.

As shown in Tables 1 to 3 the varieties put at our disposal were processed in different states of ripeness.

1.2. Preparation and Freezing

The experimental material was after cleaning blanched in water of 95 °C for 2 minutes, cooled under tap water, packed into cardboard boxes containing 0.4 kg each, quick-frozen at —30 °C and stored for 48 hours at this temperature.

1.3. Storage temperatures

The packed quick-frozen green pea samples were placed into refrigerators of $-10° \pm 4°$, $-20° \pm 1°$ and $-30° \pm 1$ °C temperature.

1.4. Test methods

The samples were exposed to physical and chemical tests and to sensory evaluation.

The peas were graded according to size and the solids content, the influence of cooking time, the enzyme activity, the alcohol insoluble solids were determined. The texture was measured by an appropriate instrument. The varieties were ranked by sensory test and the keeping quality was determined for various temperatures.

In the following paragraphs we shall describe in detail the test methods and results which served as basis for the ranking of green pea varieties after picking and storing.

1.4.1. Texture. The tenderness of green peas was determined by using the Finometer in which the peas are destroyed by a spike system. The force necessary for the destruction is related to the degree of ripeness of green peas and is indicated on the scale of the directly operating, spring actuated dynamometer. The spring constant of our Finometer was $C = 13.25$ kg/mm and it was calibrated to match the Tenderometer. By multiplying the value read off the Finometer by three we obtain the texture of grean peas in Tenderometer degrees (TÖRÖK *et al.*, 1966).

The tests were carried out on the raw peas after picking and after various treatments, including storage. The measurements were always performed at 20 °C. The frozen, i.e. stored samples were spread out at room temperature and the texture was measured when the temperature of the sample reached 20 °C as shown on the thermometer stuck among the peas. The cooked samples were cooled in cold water to 20 °C.

Five replicates were made of each measurement.

1.4.2. Alcohol insoluble solids. The alcohol insoluble solids (hereinafter: AIS) were quantitatively determined by digestion with ethylalcohol (A.O.A.C., 1960; KÖLLŐ, 1964).

1.4.3. Solids content. The solids content was determined by drying the homogenized samples to constant weight in a drying cabinet at 102 °C (Hungarian Standard MNOSz 3607—53).

1.4.4. Colour. The quantity of acetone soluble colouring matter in green peas was measured spectrophotometrically. When investigating the chlorophyll concentration with various instruments the maximum extinction values were found at different wavelengths around 600 nm (DIETRICH, 1957; 1958; KOTÁSZ, 1967).

Using a Spektromom 360 spectrophotometer we measured maximum colour intensity at 680 nm, and compared the colour of the varieties using the extinction values measured at this wavelength. The maximum was determined for the Kelvedon variety.

For the tests 1 kg of each variety was homogenized, 100 g samples were taken for each of the three parallel tests and the soluble colouring matter was extracted with acetone.

In order to compare the colour of the green pea varieties the results were expressed in percentage of the maximum extinction value taken as 100%.

1.4.5. Sensory tests. Sensory tests were performed in the Plank system with a panel consisting of the same five tasters. The various properties were scored on a 5 point scale of 1 to 5, corresponding to the terms unpalatable to excellent (SPANYÁR, 1954).

The properties tested were colour, odour, taste and texture and were weighted in accordance with their importance. Colour was multiplied by a factor of 1, odour and texture by 2, taste by 4. The scores given by the panelists were multiplied by these factors and in the evaluation these weighted values were used. Thus colour may have a maximum score of 5, odour and texture a maximum of 10, while the maximum score for taste is 20. The lower threshold of a sensorily well detectable change in quality was set at a 30% decrease in the score of taste and 50% deterioration of taste was considered the limit of keeping time.

The scored sensory results of raw green peas and of those stored under different conditions were processed by statistical methods.

The sensory scores of colour and texture were subjected to statistical analysis of deviation. Taste, being the quality primarily indicative of quality, was also evaluated by means of significance analysis. Changes occurring during storage were evaluated by comparison to fresh green peas using the t test as applied to paired samples (VINCZE, 1958) and KRAMER's method of ranking (KRAMER, 1960).

The rate of taste deterioration was expressed by the reciprocal of the time needed to reach the 30% and 50% taste deterioration ($v = 1/t$) and plotted vs. storage temperature.

1.4.6. Statistical evaluation of results. In the mathematical evaluation the following equations were used:

Standard deviation:

$$s = \sqrt{\frac{\Sigma (X - \bar{X})^2}{n - 1}}$$

where s = standard deviation
X = results of individual measurements
$\bar{X}$ = arithmetic mean of experimental results
n = number of measurements

Coefficient of variation:

$$v\% = \frac{s}{\bar{X}} \cdot 100$$

where $v\%$ = coefficient of variation.

Equation of linear regression:

$$Y' = a + bX$$

where Y' = value of the function $f(X)$
a = constant
b = constant
X = results of measurements

t test for paired data:

$$t = \frac{\bar{d}}{s_d}\sqrt{N}$$

where

$$s_d = \sqrt{\frac{\Sigma (d - \bar{d})^2}{N - 1}}$$

where d = difference between the pairs of values $X_1 - X_2$
$\bar{d}$ = arithmetic mean of differences
$d - \bar{d}$ = deviation of individual differences from the mean
N = number of paired values (half of the sum of individual measurements).

Ranking

The samples were ranked according to the scores they have been given. The sample with the highest score is ranked No 1, and the samples are put in the order of decreasing scores. Five samples thus receive rank numbers 1 to 5, and in the case of five panelists the rank numbers are repeated five times. The rank numbers of the samples are added up and the value of the best sample might be 5 (in case of 5 panelists) that of the poorest sample 25. The sum of rank numbers is evaluated with the help of Kramer's Table (KRAMER, 1960) and any significant difference in the value of a sample after storing compared to the sensory value of the same sample after picking is noted.

2. Results

2.1. Texture

The texture values of green pea varieties after picking are given in Tenderometer values in Table 1.

Changes in texture due to various treatments are shown in Figs 1 and 2.

The column graphs illustrate the textures of five green pea varieties after seven types of treatment. The group of columns marked 1, represent the

texture of the raw material, group 2 the texture of samples blanched at 95 °C for 2 minutes, group 3 that of samples blanched at 95 °C for 2 minutes, then stored at —30 °C for 12 months, while group 4 stands for the texture of samples which have been blanched at 95 °C for 2 minutes, quick-frozen and then stored at —20 °C for 12 months.

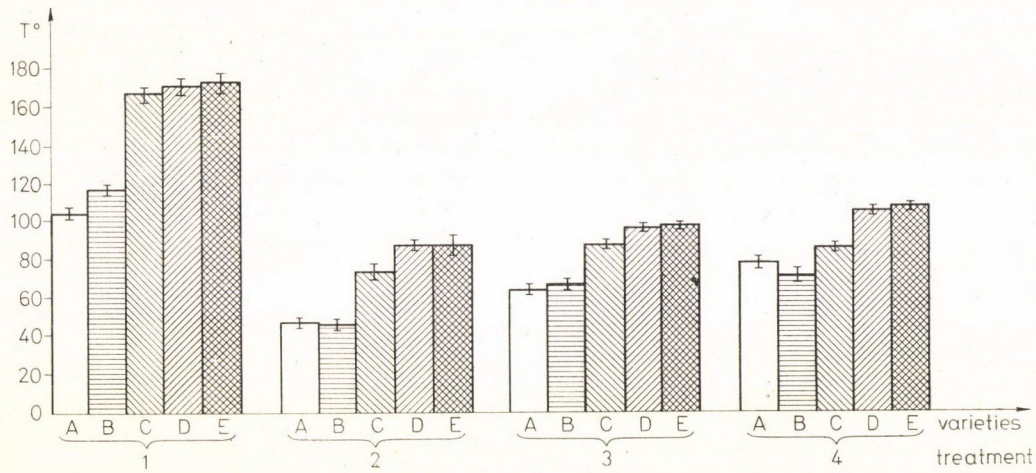

Figure 1. Texture of green pea varieties in Tenderometer degrees as a function of treatment. Texture determined with Finometer. Symbols: A = Kelvedon; B = Vitalis; C = Grüne Perle; D = Rhine dwarf; E = Mignon.1 = after picking, untreated; 2 = blanched at 95 °C for 2 minutes; 3 = blanched at 95 °C for 2 minutes, quick-frozen, stored at —30 °C for 12 months; 4 = blanched at 95 °C for 2 minutes, quick-frozen, stored at —20 °C for 12 months. The height of the columns represent the mean values, the vertical bars ± standard deviation around the mean

Table 1

Texture of green pea varieties after picking in Tenderometer degrees
(instrumental determination)

Variety	Date of picking (1969)	°T	s	v%
Kelvedon	June 20th	104.1	1.34	1.30
Vitalis	July 1st	117.3	1.64	1.40
Grüne Perle	June 26th	167.4	2.51	1.50
Rhine dwarf	June 18th	170.7	3.42	2.00
Mignon	June 11th	172.8	4.42	2.55

°T = Tenderometer degree, average of 5 measurements
s = standard deviation
v% = coefficient of variation

In Fig. 2 group I represents the texture of samples cooked at 100 °C for 9 minutes, group II that of samples blanched at 95 °C for 2 minutes, quick-frozen and stored at —30 °C for 12 months, group III that of samples blanched at 95 °C for 2 minutes, quick-frozen, stored at —20 °C for 12 months, allowed to thaw and then cooked for 5 minutes.

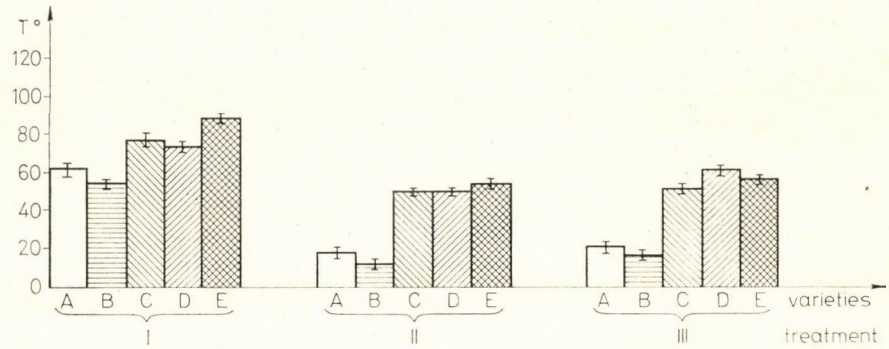

Figure 2. Texture of green pea varieties in Tenderometer degrees as a function of treatment. Texture determined with Finometer. Symbols: A = Kelvedon; B = Vitalis, C = Grüne Perle; D = Rhine dwarf; E = Mignon. I = cooked at 100 °C for 9 minutes; II = blanched at 95 °C for 2 minutes, quick-frozen, stored at —30 °C for 12 months and cooked after thawing for 5 minutes; III = blanched at 95 °C for 2 minutes, quick-frozen, stored at —20 °C for 12 months and cooked after thawing for 5 minutes. The heights of the columns represent the mean values, the vertical bars ± standard deviation around the mean

The results show the effect of various treatments on the texture of green peas. The direction and degree of textural changes depend primarily upon treatment and variety is here a negligible factor. Each variety loses by blanching at 95 °C for 2 minutes about half of its initial consistency expressed in Tenderometer degrees. Texture values after cooking at 100 °C for 9 minutes differ only slightly from the values obtained after blanching at 95 °C for 2 minutes.

Storage caused a hardening in the texture of quick-frozen green peas. Samples stored at —30 °C for 12 months showed a maximum hardening of 35%, those stored at —20 °C for the same period a maximum hardening of 66%. In the case of quick-frozen samples stored for 12 months the texture values drop suddenly after 5 minutes cooking, and compared to samples cooked after picking this decrease is about 40% in case of the harder varieties.

2.2. Alcohol insoluble solids (AIS)

Data on the alcohol insoluble solids of green pea varieties are given in Table 2.

Table 2

Alcohol insoluble solids of green peas after picking

Variety	Date of picking (1969)	Alcohol insoluble		
		%	s	v%
Kelvedon	June 20th	11.80	0.10	0.90
Vitalis	July 1st	12.60	0.30	2.10
Grüne Perle	June 26th	18.75	0.33	1.08
Rhine dwarf	June 18th	20.30	0.30	1.50
Mignon	June 11th	22.55	0.60	1.20

% = average of 3 measurements
s = standard deviation
v% = coefficient of variation

The correlation between the AIS values as shown in the Table and texture expressed in Tenderometer degrees (°T) were investigated. The results can be calculated with the help of a regression equation and expressed numerically. This regression equation is:

$$\text{AIS} = 3.2 + 0.14\,^{\circ}\text{T}$$

The value of the coefficient characteristic of the correlation is:

$$r = 0.96$$

$$N = 15$$

2.3. Solids content

The values of the solids content are shown in Table 3. Comparison of the data in the Table with the °T and AIS values indicates a correlation between the three properties. To a tender consistency belong lower AIS and solids content values.

Table 3

Solids content of green pea varieties after picking

Variety	Date of picking (1969)	Solids content		
		%	s	v%
Kelvedon	June 20th	19.91	0.10	0.50
Vitalis	July 1st	20.85	0.11	0.52
Grüne Perle	June 26th	26.22	0.17	0.65
Rhine dwarf	June 18th	26.82	0.33	1.23
Mignon	June 11th	28.88	0.22	0.76

% = average of 3 measurements
s = standard deviation
v% = coefficient of variation

2.4. Colour

The percentage values of the acetone soluble colouring matter as determined spectrophotometrically are listed in Table 4. According to these results the Mignon is the greenest variety, followed by Kelvedon and then by Grüne Perle, while Rhine dwarf and Vitalis have a duller colour.

Table 4

Colour of green pea varieties

(Measured by spectrophotometer Spektromom 360, in acetone solution, at 680 nm wavelength; cell thickness 1 cm)

Variety	Date of determination, 1969	Optical density	%	s	v%
Mignon	June 12th	0.781	100.00	0.007	0.80
Kelvedon	June 21st	0.602	76.95	0.003	0.49
Grüne Perle	June 27th	0.510	65.30	0.000	0.00
Rhine dwarf	June 19th	0.317	40.58	0.002	0.63
Vitalis	July 2nd	0.305	39.05	0.000	0.00

The extinction values are the averages of 3 samples taken always from 1 kg of green pea pulp

s = standard deviation

v% = coefficient of variation

2.5. Results of sensory tests

2.5.1. Colour tests. The differences between the colours of fresh and cold stored green pea samples were evaluated from the results of sensory tests. Figures 3 to 7 show the changes in the colour of the samples vs. storage time at various temperatures. The dashed line represents the time up to the appearance of a 30% colour deterioration.

It appears from the results that in the samples stored at —10 °C the colour deteriorates by 30% within a period of less than 4 months; at this temperature the samples showed discolouration. When stored at —20 °C, Vitalis, Mignon and Rhine dwarf varieties showed an unfavourable change, but at —30 °C no significant change was observed in the colour of any of the samples.

2.5.2. Taste testing. The results of sensory taste testing are illustrated in Figs 8 to 10, where the dashed lines represent the average values of the five varieties.

It appears from the results plotted in Fig. 8 that at —10 °C none of the varieties can be stored for more than 5 months.

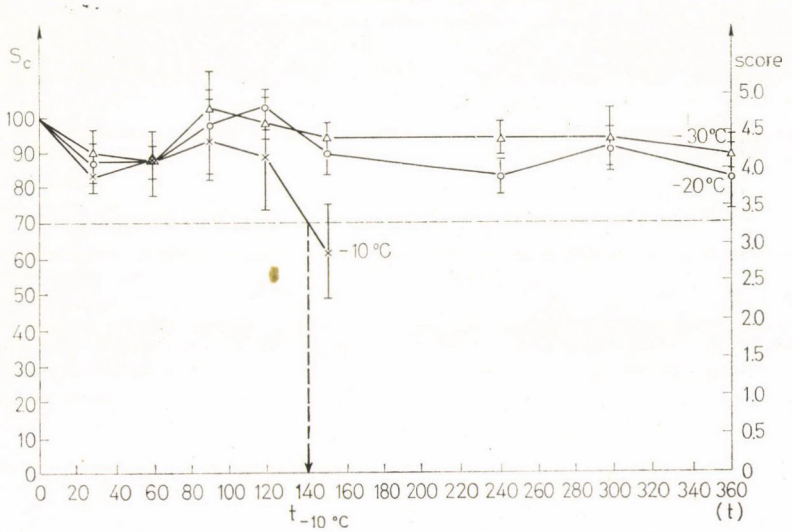

Figure 3. Changes in the colour of the Kelvedon variety vs. storage period at various temperatures. Symbols: S_c = colour score (in % of the initial value); t = storage period (days). The vertical bars represent ± standard deviation around the mean

When stored at —20 °C, with the exception of the Rhine dwarf and Mignon varieties, practically no deterioration in taste occurs for 10 months (Fig. 9). Deterioration rate increases from the 12th month onward, but only the Mignon variety suffers a sensorily detectable deterioration of 30%. According to sensory tests of the samples stored at –30 °C those which proved to have a

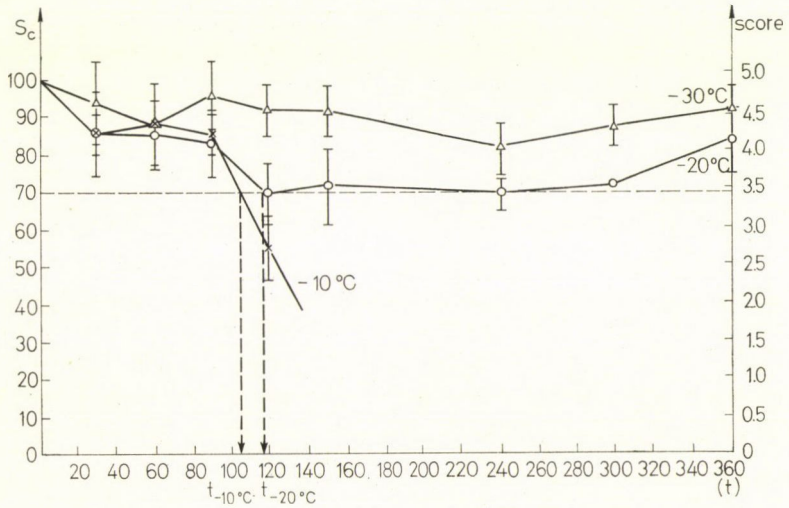

Figure 4. Changes in the colour of ± Vitalis variety vs. storage period, at various temperatures. Symbols: S_c = colour score (in % of the initial value); t = storage period (days). The vertical bars represent ± standard deviation around the mean

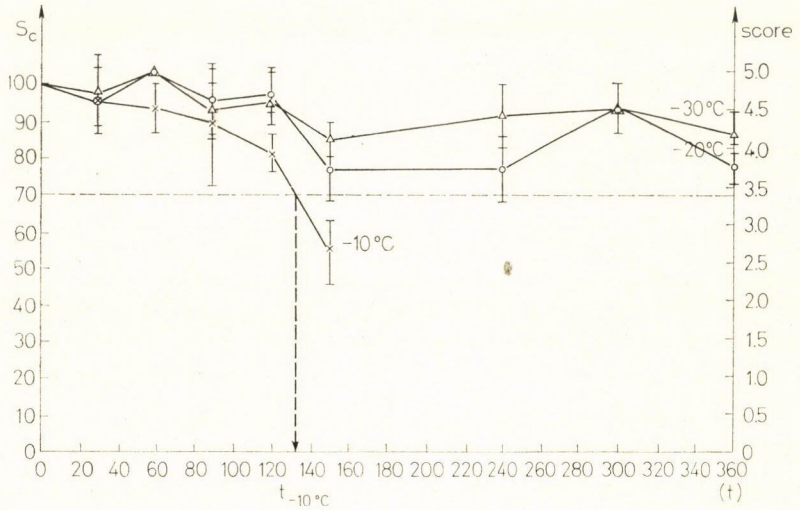

Figure 5. Changes in the colour of the Grüne Perle variety vs. storage period at various temperatures. Symbols: S_c = colour score (in % of the initial value); t = storage period (days). The vertical bars represent ± standard deviation around the mean

better quality on storage at −20 °C could be stored for a longer period. In the 10th month the average taste values of all five varieties were by 10% superior to those stored at —20 °C (Figs 9 and 10). At this temperature, too, Mignon was the only variety which suffered a 30% deterioration within 1 year.

The numerical values, together with the results of statistical analysis of deviation and significance are given in Tables 5 to 9.

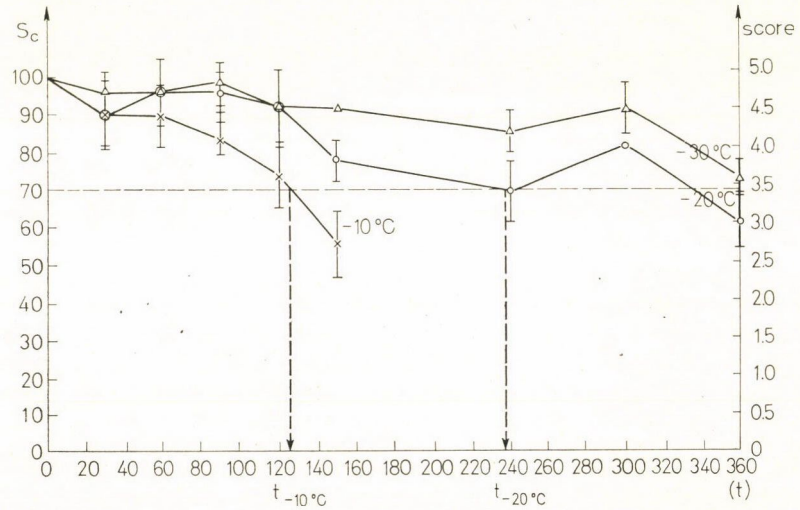

Figure 6. Changes in the colour of the Mignon variety vs. the storage period at various temperatures. Symbols: S_c = colour score (in % of the initial value); t = storage period (days). The vertical bars represent ± standard deviation around the mean

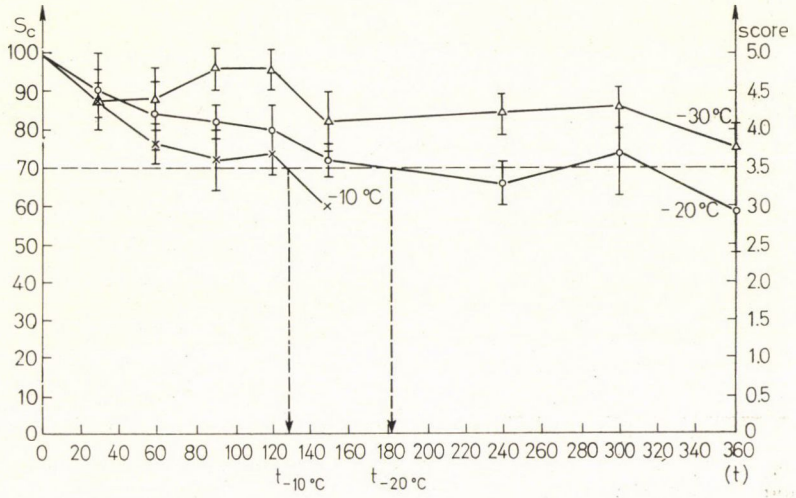

Figure 7. Changes in the colour of the Rhine dwarf variety vs. the storage period at various temperatures. Symbols: S_c = colour score (in % of the initial value); t = storage period (days). The vertical bars represent ± standard deviation around the mean

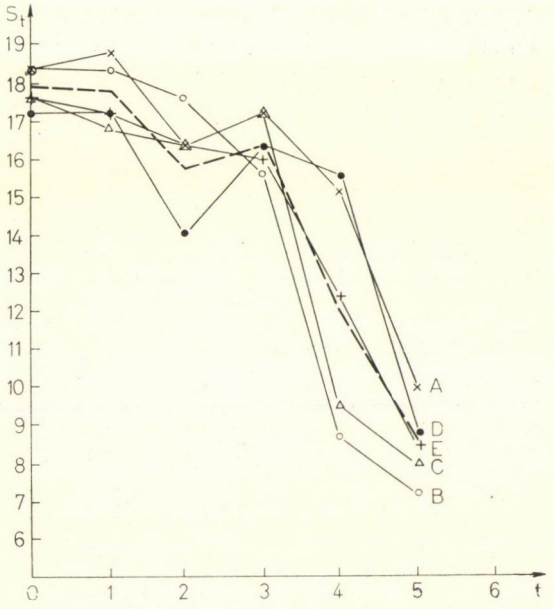

Figure 8. Changes in the taste of green peas after storage at −10 °C. Symbols: A = Kelvedon; B = Vitalis; C = Grüne Perle; D = Rhine dwarf; E = Mignon; S_t = taste score; t = storage period (months). The dashed line represents the mean values for all varieties

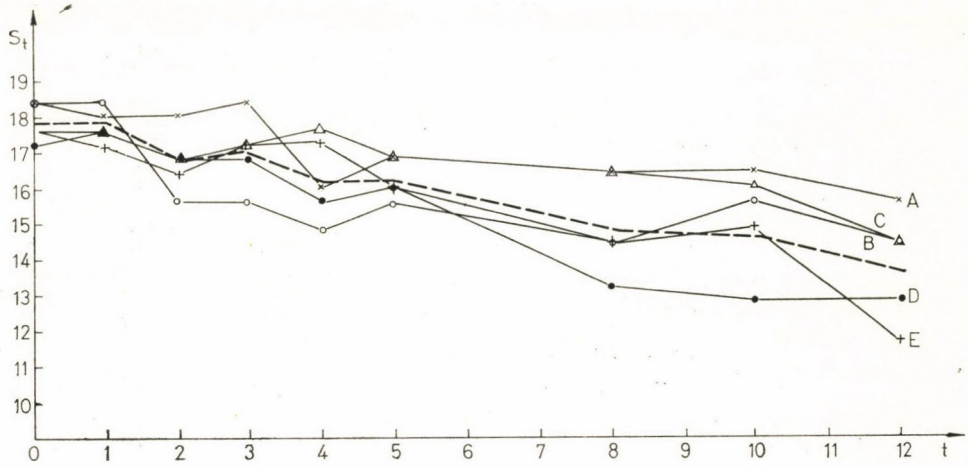

Figure 9. Changes in the taste of green peas after storage at −20 °C. Symbols: A = Kelvedon; B = Vitalis; C = Grüne Perle; D = Rhine dwarf; E = Mignon; S_t = taste score; t = storage period (months). The dashed line represents the mean values for all varieties

In significance analysis comparison is always based on the average of the sum of taste scores given by the panelists in the sensory testing of freshly picked green peas cooked for 9 minutes. According to the results shown in the Tables there was a significant or very highly significant difference between the average taste scores of samples stored at —10 °C for 5 months and those of fresh samples, independently of variety. With the exception of the Vitalis

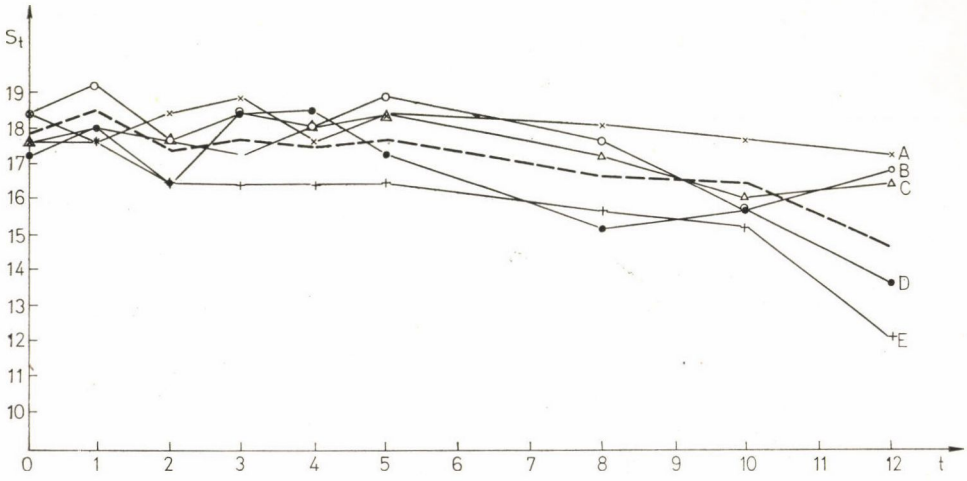

Figure 10. Changes in the taste of green peas after storage at −30 °C. Symbols: A = Kelvedon; B = Vitalis; C = Grüne Perle; D = Rhine dwarf; E = Mignon; S_t = taste score; t = storage period (months). The dashed line represents the mean values for all varieties

Table 5

Evaluation of the results of taste testing by means of the t test — KELVEDON variety

Panel of 5 tasters

Storage period months	Storage temperature								
	—10 °C			—20 °C			—30 °C		
	$\bar{x}$	s	t	$\bar{x}$	s	t	$\bar{x}$	s	t
0	18.4	1.67	—	18.4	1.67	—	18.4	1.67	—
1	18.8	1.09	0.53	18.0	1.41	0.34	17.6	1.67	0.53
2	16.4	0.89	2.24	18.0	1.41	1.00	18.4	1.67	0.00
3	17.2	2.28	0.80	18.4	0.89	0.00	18.8	1.79	0.53
4	15.2	4.60	2.36	16.0	2.82	2.06	17.6	1.60	0.99
5	10.0	2.00	7.19**	16.8	1.09	2.14	18.0	1.67	0.53
8	—	—		16.4	0.89	2.24	18.0	1.41	0.78
10	—	—		16.4	0.89	2.24	17.6	1.67	0.80
12	—	—		15.6	0.89	2.74	17.2	1.67	1.17

$\bar{x}$ = average score calculated from individual judgements
s = standard deviation of judgements
t = value of *t* test
** = highly significant at 99% probability compared to the fresh sample

Table 6

Evaluation of the results of taste testing by means of the t test — VITALIS variety

Panel of 5 tasters

Storage period months	Storage temperature								
	—10°C			—20 °C			—30 °C		
	$\bar{x}$	s	t	$\bar{x}$	s	t	$\bar{x}$	s	t
0	18.4	0.90	—	18.4	0.89	—	18.4	0.89	—
1	18.4	1.67	0.00	18.4	1.67	0.00	19.2	1.09	1.64
2	17.6	2.60	0.60	15.6	2.19	2.74	17.6	2.19	0.66
3	15.6	1.67	2.74	15.6	0.89	3.49*	18.4	1.67	0.00
4	8.8	1.10	-23.85***	14.8	2.28	3.09*	18.0	1.41	0.53
5	7.2	2.26	8.26**	15.6	1.67	2.74	18.8	1.79	2.46
8	—	—		14.4	2.19	6.34**	17.6	1.67	1.00
10	—	—		15.6	0.89	5.69**	15.6	0.89	4.26*
12	—	—		14.4	1.67	4.92**	16.8	1.09	3.58*

$\bar{x}$ = average score calculated from individual judgements
s = standard deviation between judgements
* = significant at 95% probability level compared to the fresh sample
** = highly significant at 99% probability level compared to the fresh sample
*** = very highly significant at 99.9% probability level compared to the fresh sample

Table 7

Evaluation of the results of taste testing by means of the t test — GRÜNE PERLE variety
Panel of 5 tasters

Storage period, months	Storage temperature								
	−10 °C			−20 °C			−30 °C		
	$\bar{x}$	s	t	$\bar{x}$	s	t	$\bar{x}$	s	t
0	17.6	2.61	—	17.6	2.61	—	17.6	2.61	—
1	16.8	1.09	0.78	17.6	1.67	0.00	18.0	1.41	0.40
2	16.4	1.41	0.68	16.8	1.79	0.78	17.6	2.19	0.00
3	17.2	1.79	0.34	17.2	1.79	0.27	17.2	1.79	0.27
4	9.6	1.67	8.94***	17.6	0.89	0.00	18.0	2.00	0.30
5	8.0	1.41	9.80***	16.8	1.09	0.53	18.4	1.67	0.53
8	—	—		16.4	0.89	0.80	17.2	1.09	0.30
10	—	—		16.0	1.41	1.63	16.0	1.41	0.66
12	—	—		14.8	2.28	1.29	16.4	1.67	1.29

$\bar{x}$ = average score calculated from individual judgements
s = standard deviation between judgements
t = value of *t* test
*** = very highly significant at 99.9% probability level compared to the fresh sample

Table 8

Evaluation of the results of taste testing by means of the t test — RHINE DWARF variety
Panel of 5 tasters

Storage period, months	Storage temperature								
	−10 °C			−20 °C			−30 °C		
	$\bar{x}$	s	t	$\bar{x}$	s	t	$\bar{x}$	s	t
0	17.2	1.79	—	17.2	1.79	—	17.2	1.79	—
1	17.2	1.09	0.00	17.6	1.67	1.00	18.0	1.41	0.59
2	14.0	1.41	6.59**	16.8	1.79	0.40	16.4	1.67	0.66
3	16.4	0.89	0.99	16.8	1.09	0.53	18.4	0.89	1.17
4	15.6	1.67	2.14	15.6	0.89	1.63	18.4	0.89	1.17
5	8.8	1.79	11.24***	16.0	2.82	0.88	17.2	1.09	0.00
8	—	—		13.2	1.79	6.34**	15.2	1.09	3.14*
10	—	—		12.8	1.09	3.78*	15.6	0.89	1.38
12	—	—		12.8	1.09	4.49*	13.6	0.89	4.82**

$\bar{x}$ = average score calculated from individual judgements
s = standard deviation between judgements
t = value of *t* test
* = significant at 95% probability level compared to the fresh sample
** = highly significant at 99% probability level compared to the fresh sample
*** = very highly significant at 99.9% probability level compared to the fresh sample

Table 9

Evaluation of the results of taste testing by means of the t test — MIGNON variety

Panel of 5 tasters

Storage period, months	Storage temperature								
	—10 °C			—20 °C			—30 °C		
	$\bar{x}$	s	t	$\bar{x}$	s	t	$\bar{x}$	s	t
0	17.6	0.89	—	17.6	0.89	—	17.6	0.89	—
1	17.2	1.09	0.99	17.2	1.79	0.53	17.6	0.89	2.43
2	16.4	1.67	1.49	16.4	0.89	2.43	16.4	1.67	1.49
3	16.0	1.41	2.14	17.2	1.09	0.53	16.4	0.89	2.46
4	12.4	0.89	10.57***	17.2	1.09	0.53	16.4	2.19	1.00
5	8.4	1.67	18.70***	16.0	1.41	2.14	16.4	1.67	1.17
8	—	—		14.4	2.60	3.13*	15.6	1.67	2.24
10	—	—		14.8	1.09	3.49*	15.2	1.09	5.96**
12	—	—		11.6	1.67	6.71**	12.0	0.00	10.66***

$\bar{x}$ = average score calculated from individual judgements
s = standard deviation between judgements
t = value of *t* test
* = significant at 95% probability level compared to the fresh sample
** = highly significant at 99% probability level compared to the fresh sample
*** = very highly significant at 99.9% probability level compared to the fresh sample

variety, samples stored at —20 °C showed a significant deterioration only after 8 months storage. The average results of samples of all varieties are better when stored at —30 °C, but here, too, significant differences were observed.

2.6. Deterioration rate

The deterioration rates of the green pea varieties under investigation were calculated from the results of sensory taste testing. Deterioration rate was related to 30% taste deterioration as the threshold value and to the practically still acceptable value of 50% taste deterioration.

The rate of 30% taste deterioration is plotted in Fig. 11 as the function of temperature.

The keeping times calculated from 30% taste deterioration are favourable at storage temperatures of —20° and —30 °C. It should be borne in mind that 30% taste deterioration specified by us does not represent the limit of keeping time, only a failure of the sample to comply with the requirements of first class quality. From the time-temperature graph the following keeping time results can be concluded:

Variety	Keeping time in days		
	at —10 °C	at —20 °C	at —30 °C
Kelvedon	133	435	480
Vitalis	102	402	468
Grüne Perle	109	433	480
Mignon	121	338	351
Rhine dwarf	135	384	408

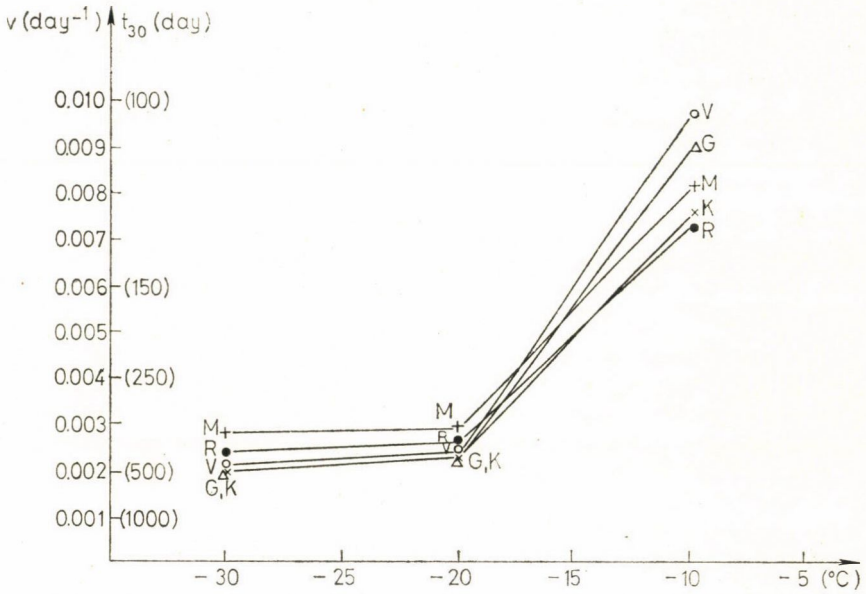

Figure 11. Deterioration rate (v) accounting for a 30% deterioration of taste, vs. storage temperature. Symbols: K = Kelvedon; V = Vitalis; G = Grüne Perle; R = Rhine dwarf; M = Mignon

It appears from the results that storage at —10 °C should be applied for a very short period only. At —20 °C and —30 °C green pea samples can be stored without any significant loss in quality for almost the same periods. No significant difference was found between the time-temperature tolerance of samples of different varieties when stored at —20° and —30 °C, resp.

The 50% loss in taste, which marks practically the end of keeping time is shown in Fig. 12.

The keeping times determined from the results are as follows:

Variety	Keeping time in days		
	at —10 °C	at —20 °C	at —30 °C
Kelvedon	163	610	673
Vitalis	118	563	657
Grüne Perle	135	605	670
Mignon	147	474	490
Rhine dwarf	153	535	569

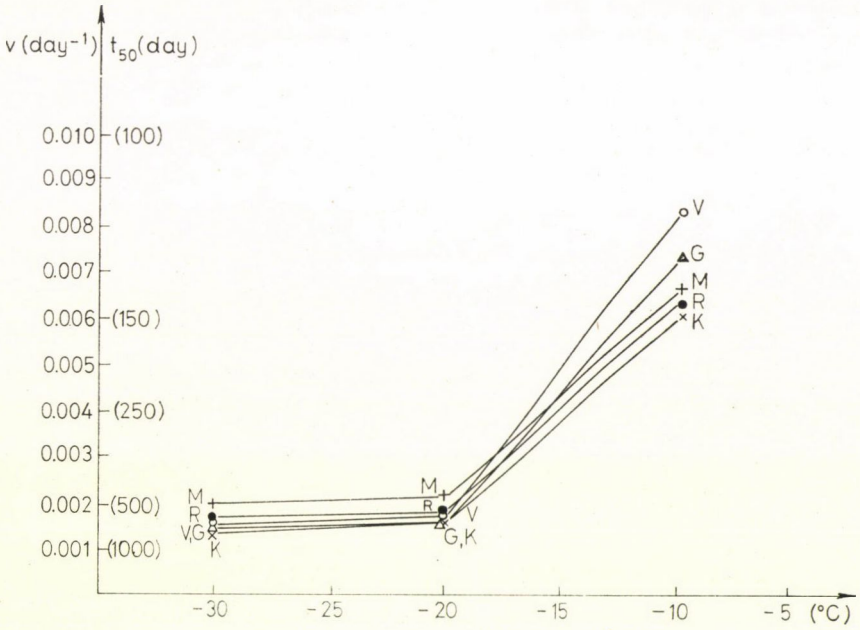

Figure 12. Deterioration rate (v), accounting for a 50% deterioration of taste, vs. storage temperature. Symbols: **K** = Kelvedon; **V** = Vitalis; **G** = Grüne Perle; **R** = Rhine dwarf; **M** = Mignon

It appears from the comparison of the data that the first three varieties can be stored at —20 °C and —30 °C for almost two years. No significant differences were found in the comparison of the varieties and of the two storage temperatures. Storage at —10 °C ensures a storage period of maximum 5 months, even if 50% loss is considered.

The period between 30% and 50% deterioration in taste was analyzed in relation to variety and storage temperature. The aim of this analysis was to establish whether the differences in the periods which elapse between the first detectable loss in taste (30%) and the keeping time limit (50% deterioration) were significant. The differences were found significant in every case

and there was also a difference in the level of significance. The results of the t test are illustrated by the following data:

Varieties	t values of the differences between the periods which elapse from 30% to 50% deterioration	
	−20 °C	−30 °C
Kelvedon	7.65 **	9.02 ***
Vitalis	7.52 **	18.80 ***
Grüne Perle	3.18 *	4.25 *
Rhine dwarf	6.77 **	10.99 ***
Mignon	4.92 **	16.22 ***

Significance levels were determined by analyzing the t values for 4 degrees of freedom. A markedly higher significance level was shown at −30 °C than at −20 °C. This indicates a lower deterioration rate at −30 °C which is the natural consequence of storage at lower temperature.

The relative rate of deterioration was calculated from the function B/T^2 and was found to be 0.3 to 0.4 K^{-1} where B approximately corresponds to the quotient of activation energy and of the universal gas constant and T is the absolute temperature.

3. Conclusions

It has been established from the experimental results that the green pea varieties under investigation can be ranked according to KRAMER (1960) based on the taste scores, as follows:

1. Kelvedon
2. Grüne Perle
3. Vitalis
4. Rhine dwarf
5. Mignon

For quick-freezing followed by prolonged cold storage the Kelvedon and Grüne Perle, and then the Vitalis variety are suitable.

Special attention is drawn to the variety Grüne Perle which, in spite of the unfavourable initial texture test results, due to a rather unsuitable date of picking, showed favourable storage properties all along.

The storage temperature of −20 °C was found suitable for periods shorter than 18 months. However, according to the results for longer periods storage at −30 °C should be preferred since at this temperature storage time may be extended by 1 to 2 months (5 to 10%). Similar results on time-temperature tolerance were obtained by JUL (1961).

A close correlation was established between texture and the alcohol insoluble solids, as supported by several earlier publications.

*

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